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09/121 798
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1. Document ID: US 6197553 B1

L8: Entry 1 of 43

File: USPT

Mar 6, 2001

US-PAT-NO: 6197553
DOCUMENT-IDENTIFIER: US 6197553 B1
TITLE: Method for large scale plasmid purification
DATE-ISSUED: March 6, 2001

US-CL-CURRENT: 435/91.1; 424/184.1, 435/259, 435/306.1, 435/320.1, 514/44, 536/23.1, 536/25.4

APPL-NO: 8/ 952428
DATE FILED: November 7, 1997

PARENT-CASE:
RELATED APPLICATION This is a 35 U.S.C. .sctn.371 U.S. national application of PCT/US96/07083,
filed May 15, 1996, which is a continuation-in-part of U.S. application Ser. No. 08/446,118,
filed May 19, 1995, now abandoned, which is a continuation-in-part of U.S. application Ser. No.
08/275,571, filed Jul. 15, 1994, now abandoned.

IN: Lee; Ann L, Sagar; Sangeetha

AB: A process is disclosed for the large scale isolation and purification of plasmid
DNA from large scale microbial fermentations. The process exploits a rapid heating method to
induce cell lysis and precipitate genomic DNA, proteins and other debris while keeping the
plasmid in solution. Suspending the microbial cells in buffer and then heating the
suspension to about 70-100.degree. C. in a flow-through heat exchanger results in excellent
lysis. Continuous flow or batch-wise centrifugation of the lysate effects a pellet that
contains the cell debris, protein and most of the genomic DNA while the plasmid remains in
the supernatant. This invention offers a number of advantages including higher product
recovery than by chemical lyses, inactivation of Dnases, operational simplicity and
scaleability.

L8: Entry 1 of 43

File: USPT

Mar 6, 2001

DOCUMENT-IDENTIFIER: US 6197553 B1
TITLE: Method for large scale plasmid purification

BSPR:
The current laboratory method used to isolate and purify plasmid DNA consists of a series of
classical laboratory techniques that are not suitable for a manufacturing process. For example,
density gradient centrifugations are not scaleable; the purification procedure necessitates the
use of hazardous and expensive chemicals/solvents such as ethydium bromide, a known mutagen, and
is labor intensive and time consuming. Therefore, a scaleable alternative process was developed,
and is disclosed herein. In addition, an HPLC assay was established to track the plasmid product
through the process steps and to distinguish between the plasmid forms. The microbial cells
harboring the plasmid are suspended and optionally incubated with lysozyme in a buffer containing
detergent, heated using a flow-through heat exchanger to lyse the cells, followed by
centrifugation. After centrifugation the clarified lysate, which contains predominately RNA and
the plasmid product, is filtered through a 0.45 micron filter and then diafiltered, prior to
loading on the anion exchange column. The plasmid product may optionally be treated with RNase
before or after filtration, or at an earlier or later step. The anion exchange product fraction
containing the plasmid is loaded onto the reversed phase column, and is eluted with an
appropriate buffer, providing highly pure plasmid DNA suitable for human use.

DEPR:
The eluted plasmid DNA can then be concentrated and/or diafiltered to reduce the volume or to
change the buffer. For DNA intended for human use it may be useful to diafilter the DNA product
into a pharmaceutically acceptable carrier or buffer solution. Pharmaceutically acceptable
carriers or buffer solutions are known in the art and include those described in a variety of
texts such as Remington's Pharmaceutical Sciences. Any method suitable for concentrating a DNA
sample is suitable for use in the present invention. Such methods includes diafiltration, alcohol
precipitation, lyophilyzation and the like, with diafiltration being preferred. Following
diafiltration the final plasmid DNA product may then be sterilized. Any method of sterilization
which does not affect the utility of the DNA product is suitable, such as sterilization by
passage through a membrane having a sufficiently small pore size, for example 0.2 microns and
smaller.

CLPV:
e) filtering and diafiltering the supernatant of step d) to provide a filtrate containing the
plasmid DNA;

CLPV:
e) filtering and diafiltering the supernatant of step d) to provide a filtrate containing the
plasmid DNA;

2. Document ID: US 6150586 A

L8: Entry 2 of 43

File: USPT

Nov 21, 2000

US-PAT-NO: 6150586
DOCUMENT-IDENTIFIER: US 6150586 A
TITLE: Plant gene encoding acetyl coenzyme a carboxylase biotin carboxyl carrier protein
DATE-ISSUED: November 21, 2000

US-CL-CURRENT: 800/281; 435/419, 435/468, 536/23.6, 800/286, 800/298

APPL-NO: 8/ 983409
DATE FILED: January 20, 1998

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9516961	August 18, 1995

PCT-DATA:
APPL-NO
DATE-FILED
PUB-NO
PUB-DATE
371-DATE
102(E)-DATE
PCT/GB96/01894
August 6, 1996
WO97/07222
Feb 27, 1997
Jan 20, 1998
Jan 20, 1998

IN: Slabas; Antoni Ryszard, Elborough; Kieran Michael

AB: The capacity of a plant to produce fatty acids is modulated by controlling the
expression of a gene specifying the acetyl CoA carboxylase biotin carboxyl carrier protein.
Modulation may comprise increasing the expression of the gene by insertion of additional
copies into the genome or inhibiting expression by insertion of an antisense or
cosuppression vector directed against the endogenous gene.

L8: Entry 2 of 43
File: USPT
Nov 21, 2000

DOCUMENT-IDENTIFIER: US 6150586 A
TITLE: Plant gene encoding acetyl coenzyme a carboxylase biotin carboxyl carrier protein

DEPR:
cDNA probes for screening the rape libraries were generated by the appropriate restriction
endonuclease digestions of plasmid DNA. The DNA fragment required was separated from vector DNA
by TAE agarose electrophoresis and isolated using the GeneClean II kit (Bio 101) or by freezing
and ultrafiltration.

3. Document ID: US 6110665 A

L8: Entry 3 of 43
File: USPT
Aug 29, 2000

US-PAT-NO: 6110665
DOCUMENT-IDENTIFIER: US 6110665 A
TITLE: Sarcocystis neuronadiagnostic primer and its use in methods of equine protozoal
myeloencephalitis diagnosis
DATE-ISSUED: August 29, 2000

US-CL-CURRENT: 435/6; 435/91.2, 536/24.32, 536/24.33

APPL-NO: 8/ 388029
DATE FILED: February 14, 1995

IN: Fenger; Clara K., Granstrom; David E., Gajadhar; Alvin A., Dubey; Jitender P.

AB: An amplification primer and probe which can be used in an in vitro diagnostic
test for the presence of S. neurona in equine blood or cerebrospinal fluid. Sarcocystis
neurona is responsible for the equine condition of protozoal myelitis. The amplification
primer is seventeen nucleotides in length and complementary to a unique section of the small
ribosomal subunit of Sarcocystis neurona. The primer encompasses nucleotide positions
1470-1487 of the small ribosomal subunit of S. neurona. The primer has the sequence 5'
CCATTCCGGACGCGGGT SEQ ID NO:1.

L8: Entry 3 of 43
File: USPT
Aug 29, 2000

DOCUMENT-IDENTIFIER: US 6110665 A
TITLE: Sarcocystis neuronadiagnostic primer and its use in methods of equine protozoal
myeloencephalitis diagnosis

DEPR:
The PCR product was purified by ultrafiltration (Krowczynska and Henderson, 1992) using
microcon-100 microconcentrators (Amicon, Beverly, Mass.). The amplified product was directly
ligated into a pT7Blue vector (Novagen, Madison, Wis.) and transformed according to the
recommendations of the manufacturer. Transformed cells were selected by culturing on LB agar
plates containing 50 .mu.g/ml ampicillin and 15 .mu.g/ml tetracycline. Colonies containing PCR
product insert were selected using blue/white screening, by the addition of 40 .mu.l of 20 mg/ml
X-gal in dimethyl formamide, and 4 .mu.l 200 mg/ml IPTG per 100 mm plate. Plates were incubated
for 12 to 24 hours (Sambrook, Fritsch and Maniatis, 1989). White colonies were screened for
insert by PCR. Individual colonies were scraped from the plate, and diluted in 20 .mu.l of
sterile water in 1.5 ml microcentrifuge tubes. These were boiled for 2 min to release plasmid
DNA, and 10 .mu.l of supernatant was used in the PCR protocol described above. Twelve positive
clones were identified in this manner, and two were arbitrarily chosen for sequencing.

4. Document ID: US 6093701 A

L8: Entry 4 of 43
File: USPT
Jul 25, 2000

US-PAT-NO: 6093701
DOCUMENT-IDENTIFIER: US 6093701 A
TITLE: Method for covalent attachment of compounds to genes
DATE-ISSUED: July 25, 2000

US-CL-CURRENT: 514/44; 435/320.1, 435/325, 435/455, 435/69.1, 536/23.1

APPL-NO: 8/ 990015
DATE FILED: December 12, 1997

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS (Provisional

Application Ser. No. 60/050,842) (Filing Date Jun. 26, 1997)

IN: Wolff; Jon A., Hagstrom; James E., Sebestyen; Magdolna G., Budker; Vladimir

AB: The described invention relates to methods for covalently attaching a compound to a gene. The method provides for covalently attaching compounds to genes for enhancing the cellular transport of the genes to predetermined targets, while maintaining the gene's functionality.

L8: Entry 4 of 43 File: USPT Jul 25, 2000

DOCUMENT-IDENTIFIER: US 6093701 A
TITLE: Method for covalent attachment of compounds to genes

DEPL: Preparation of fluorescently labeled DNA--Primary amine groups were introduced into double stranded DNA by two alternative approaches. 4-(N-2-chloroethyl-N-methylamino)-benzylamine RCl) (kindly provided by A. Mustaev) alkylates guanines at the N7 ring nitrogen (Grineva, N., Knorre, D. and Kurbatov, V. 1971. Highly efficient alkylation of transport RNA by 4-(N-2-chloroethyl-N-methylamino)benzylamine. Doklady Akademii Nauk SSSR 201:609-611.). It was dissolved in dimethylformamide (DMF) and mixed with double stranded DNA (2 .mu.g/.mu.l final concentration) at 1:6 RCl to nucleotide molar ratio, in PBS containing 25% DMF. Reactions were incubated overnight at room temperature (RT) followed by gel filtration on Sephadex G-25 (NAP-5 columns; Pharmacia). The amine-modified DNA was concentrated in an Ultrafree-MC 30,000 NMWL ultrafiltration unit (Millipore). Another approach involved the use of 4-(phenyl-azido-salicyl-amido)-butylamine (ASBA) (Pierce) which reacts with nucleophilic groups of the DNA after photoactivation. ASBA was dissolved in PBS and added to the DNA (1.2 .mu.g/.mu.l final DNA concentration) at a molar ratio of 1.3:1 ASBA to nucleotide. The samples were UV illuminated as previously described (Dowty, M. E., Guervich, V., Berg, R. K., Repetto, G. and Wolff, J. A. 1992. Characterization of biotinylated and gold labeled plasmid DNA. Meth. Molec. Cell. Biol. 3:167-174.). Excess ASBA was removed and the DNA was concentrated as above.

5. Document ID: US 6074873 A

L8: Entry 5 of 43 File: USPT Jun 13, 2000

US-PAT-NO: 6074873
DOCUMENT-IDENTIFIER: US 6074873 A
TITLE: Nucleic acids encoding NL-3
DATE-ISSUED: June 13, 2000

US-CL-CURRENT: 435/325; 435/252.3, 435/254.11, 435/320.1, 435/69.1, 530/350, 536/23.1, 536/23.5

APPL-NO: 9/ 143068
DATE FILED: August 28, 1998

PARENT-CASE: This is a continuation-in-part of co-pending application(s) Ser. No. 08/934,494 filed on Sep. 19, 1997, to which application(s) priority is claimed under 35 USC .sctn.120.

IN: Fong; Sherman, Ferrara; Napoleone, Goddard; Audrey, Godowski; Paul J., Gurney; Austin L., Hillan; Kenneth, Williams; P. Mickey

AB: The present invention concerns isolated nucleic acid molecules encoding the novel TIE ligand homologues NL2, NL3 andNL6 (FLS139), the proteins encoded by such nucleic acid molecules, as well as methods and means for making and using such nucleic acid and protein molecules.

L8: Entry 5 of 43 File: USPT Jun 13, 2000

DOCUMENT-IDENTIFIER: US 6074873 A
TITLE: Nucleic acids encoding NL-3

DEPR: The ampoule containing NL2, NL3 or NL6 plasmid DNA was thawed by placement into water bath and mixed by vortexing. The contents were pipetted into a centrifuge tube containing 10 mLs of medium and centrifuged at 1000 rpm for 5 minutes. The supernatant was aspirated and the cells were resuspended in 10 mL of selective medium (0.2 Mm filtered PS20 with 5% 0.2 .mu.m diafiltered fetal bovine serum). The cells were then aliquoted into a 100 mL spinner containing 90 mL of selective medium. After 1-2 days, the cells were transferred into a 250 mL spinner filled with 150 mL selective growth medium and incubated at 37.degree. C. After another 2-3 days, a 250 mL, 500 mL and 2000 mL spinners were seeded with 3.times.10.sup.5 cells/mL. The was exchanged with fresh medium by centrifugation and resuspension in production medium. Any suitable CHO medium may be employed, e.g., such as is described in U.S. Pat. No. 5,122,469, issued Jun. 16, 1992. A 3 L production spinner is seeded at 1.2.times.10.sup.6 cells/mL. On day 0, the cell number and pH were determined. On day 1, the spinner was sampled and sparging with filtered air was commenced. On day 2, the spinner was sampled, the temperature shifted to 33.degree. C., and 30 mL of 500 g/L-glucose and 0.6 mL of 10% antifoam (e.g., 35% polydimethylsiloxane emulsion, Dow Corning 365 Medical Grade Emulsion) were added. Throughout the production, pH was adjusted as necessary to keep at around 7.2. After 10 days, or until viability dropped below 70%, the cell culture was harvested by centrifugation and filtered through a 0.22 .mu.m filter. The filtrate was either stored at 4.degree. C. until loading onto a purification column.

6. Document ID: US 6030616 A

L8: Entry 6 of 43

File: USPT Feb 29, 2000

US-PAT-NO: 6030616
DOCUMENT-IDENTIFIER: US 6030616 A
TITLE: Hepatitis B escape mutant specific binding molecules
DATE-ISSUED: February 29, 2000

US-CL-CURRENT: 424/149.1; 424/130.1, 424/161.1, 435/339, 435/346, 435/5, 435/69.1, 435/7.1, 435/70.21, 435/975, 530/388.3

APPL-NO: 8/ 519981
DATE FILED: August 28, 1995

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9306087	March 24, 1993
GB	9311526	June 4, 1993
WO	PCT/GB94/00609	March 24, 1994

IN: Waters; Jennifer Anne, Carman; William Frederick, Thomas; Howard Christopher

AB: Molecules which are capable of specifically binding to a hepatitis B escape mutant antigenic determinant include monoclonal antibodies secreted by the cell line SMH HBs 145/G/R/I (ECACC 92122312). SMH HBs 145/R/I (ECACC 93052626). SMH HBs 145/G/II (ECACC 93033109) or SMH HBs 145/R/II (ECACC 93033110) and other specific binding molecules cross-competitive with them. Antibodies secreted by the cell lines SM HBs 145/G/R/I and SMH HBs 145/G/R/II bind variant (escape mutant) HBsAG and wild type HBsAG. Antibodies secreted by the cell lines SMH HBs 145/R/I and SMH HBs 145/R/II bind variant but not wild type.

L8: Entry 6 of 43 File: USPT Feb 29, 2000

DOCUMENT-IDENTIFIER: US 6030616 A
TITLE: Hepatitis B escape mutant specific binding molecules

DEPR:
Yeast strain DC5 cir.degree. was transformed with DNA of plasmid pRIT13557 to establish strain Y1648, as described in Example 2 or WO-A-9114703. Strain Y1648 expresses variant HBsAg, with a Gly.fwdarw.Arg mutation at position 145. Variant HBsAg was isolated from a culture (designated C1334) of strain Y1648 by AEROSIL.TM. adsorption/desorption, ultrafiltration, ion-exchange column chromatography, CsCl density gradient centrifugation and dialysis of the CsCl gradient fractions.
The batch of purified antigen was designated 31M5.

7. Document ID: US 6011148 A

L8: Entry 7 of 43 File: USPT Jan 4, 2000

US-PAT-NO: 6011148
DOCUMENT-IDENTIFIER: US 6011148 A
TITLE: Methods for purifying nucleic acids
DATE-ISSUED: January 4, 2000

US-CL-CURRENT: 536/25.4; 435/91.1

APPL-NO: 8/ 691090
DATE FILED: August 1, 1996

IN: Bussey; Lee B., Adamson; Robert, Atchley; Alan

AB: Methods are provided for producing highly purified compositions of nucleic acids by using tangential flow ultrafiltration. A scaleable process for producing pharmaceutical grade plasmid DNA, useful for gene therapy, is provided, which is efficient and avoids the use of toxic organic chemicals.

L8: Entry 7 of 43 File: USPT Jan 4, 2000

DOCUMENT-IDENTIFIER: US 6011148 A
TITLE: Methods for purifying nucleic acids

ABPL:
Methods are provided for producing highly purified compositions of nucleic acids by using tangential flow ultrafiltration. A scaleable process for producing pharmaceutical grade plasmid DNA, useful for gene therapy, is provided, which is efficient and avoids the use of toxic organic chemicals.

BSPR:
In preferred embodiments, the nucleic acid is DNA, particularly viral or plasmid DNA. The ultrafiltration unit is preferably an open-channel, flat plate device. In further preferred embodiments, the ultrafiltration membrane has a molecular weight cut-off of between 1 K and 1,000 K, most preferably around 300 K. The method preferably includes preparing the gel layer using pressure of from about 5 psi to about 30 psi, preferably around 10 to 15 psi. The nucleic acid solution may be concentrated in the range of about 2-fold to about 50-fold during the ultrafiltration step.

DEPR:
Initial preparatory purification of the nucleic acid sample before tangential flow ultrafiltration will depend on the source of the nucleic acid and the level of purity desired.
Ideally, many contaminants are removed by one or more coarse purification steps before tangential flow ultrafiltration to reduce the number of contaminating particles that could foul the ultrafiltration membrane, impeding performance, and decrease the amount of any larger contaminants that would be retained with the nucleic acid. For nucleic acids obtained from biological sources, e.g. tissues and cells, including cell lines, mammalian, yeast, plant or bacterial cells, initial preparatory steps to lyse cells and remove cell

components, e.g.
proteins, cell walls or membranes, can be performed using conventional methods known to those of
ordinary skill in the art. See, e.g., Sambrook et al., 1989; Ausubel et al., 1989. For
purification of extrachromosomal DNA, such as plasmid DNA, it is desirable to use methods that do
not shear chromosomal DNA, making its removal simpler and avoiding contamination with the final
plasmid DNA product. Thus, for example, plasmid DNA may be isolated from bacterial sources using
conventional procedures including lysis with alkali and/or detergents, e.g. SDS, NP40, Tween 20
and the like, mechanical methods, or boiling, followed by precipitation of proteins, chromosomal
DNA and cell debris. (see Sambrook, et al., 1989; Carlson et al., 1995, Biotech. Bioeng. 48:
303-315; Hirt, 1967, J. Mol. Biol. 26: 365-369) For purification of extrachromosomal DNA from
mammalian cells, e.g., a conventional Hirt extraction may be used. Sambrook et al., 1989; Ausubel
et al., 1989. For synthetic nucleic acids, little or no pretreatment may be necessary before TFU.

DEPR:
The ultrafiltration membrane will be selected based on the size and conformation of the nucleic
acid to be purified, and typically will have a molecular weight cut-off (MWCO) in the range of 1
K to 1,000 K daltons. For many supercoiled plasmid DNAs, ultrafiltration membranes having a MWCO
around 300 K daltons may be used. For some larger plasmids, however, improved speed, purity and
quality of the resultant DNA is obtained when larger MWCO membranes are used. Preferably,
therefore, plasmid DNA with sizes ranging from about 2 Kb to 15 Kb are purified using
ultrafiltration membranes having a MWCO of 300 K daltons; plasmids ranging from about 15 Kb to
about 50 Kb may be purified using membranes having a MWCO of 500 K daltons; and plasmids of about
40 Kb or larger may be purified using membranes having a MWCO of 1,000 daltons. Under these
conditions, plasmid DNA will be retained in the retentate while contaminating substances
including many proteins, cell membrane debris, carbohydrates, small degraded nucleotides, etc.,
pass through the membrane into the filtrate. Smaller nucleic acids, e.g., small synthetic
oligonucleotides, may be purified using ultrafiltration membranes with a MWCO of around 1 K to 5
K daltons. For any nucleic acid to be purified, the optimal membrane pore size may be determined
empirically using small scale devices, e.g., centrifugation devices or stirred cell devices,
available from a variety of commercial manufacturers. A manifold system may be used for
optimizing parameters in process scale development. Commercial sources for ultrafiltration
devices include Pall-Filtron (Northborough, Mass.), Millipore (Bedford, Mass.), and Amicon
(Danvers, Mass.).

DEPR:
Yield of final plasmid DNA product from the final ultrafiltration was 80%. The final product was
then aliquotted and stored at -20.degree. C. until use. The final product was determined to meet
the following Quality Control specifications:

CLPV:
b) filtering the solution through the ultrafiltration unit comprising a gel layer to provide a
permeate solution and a retentate solution whereby the plasmid DNA is retained in the retentate
solution;

CLPV:
b) filtering the solution through the ultrafiltration unit comprising a gel layer to provide a
retentate solution and a permeate solution whereby the plasmid DNA is retained in the retentate
solution;

CLPV:
e) further purifying the plasmid DNA by filtering the substantially purified plasmid DNA solution
by tangential flow ultrafiltration to provide a permeate solution and a retentate solution
whereby the plasmid DNA is retained in the retentate solution;

CLPV:
a) filtering the solution through an open-channel ultrafiltration unit comprising a membrane
having a molecular weight cutoff in the range of from about 50 K to about 500 K daltons to
provide a permeate solution and a retentate solution, whereby the plasmid DNA is retained in the
retentate solution; and

CLPV:
b) filtering the solution through the ultrafiltration unit comprising a gel layer to provide a
retentate solution and a permeate solution whereby the plasmid DNA is retained in the retentate
solution;

8. Document ID: US 5981735 A

L8: Entry 8 of 43 File: USPT Nov 9, 1999

US-PAT-NO: 5981735
DOCUMENT-IDENTIFIER: US 5981735 A
TITLE: Method of plasmid DNA production and purification
DATE-ISSUED: November 9, 1999

US-CL-CURRENT: 536/25.4; 424/124, 435/384, 435/404, 530/417, 536/26.42, 536/26.43, 71/8

APPL-NO: 8/ 798825
DATE FILED: February 12, 1997

PARENT-CASE:
This application is a continuation of U.S. provisional application Ser. No. 60/012,736, filed
Mar. 4, 1996, and now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
GB	9602825	February 12, 1996

IN: Thatcher; David R., Hitchcock; Anthony, Hanak; Julian A.J., Varley; Diane L.

AB: A scalable method for the production of highly purified plasmid DNA in
Escherichia coli is described, which method includes growing plasmid-containing cells to a
high biomass in exponential growth and lysing the cells by raising the pH of the culture to

a carefully controlled pH value in which chromosomal DNA is denatured but plasmid DNA is reversibly renatured. The method has been developed for the production of pharmaceutical grade DNA for use in in vivo and ex vivo gene therapy.

L8: Entry 8 of 43 File: USPT Nov 9, 1999

DOCUMENT-IDENTIFIER: US 5981735 A
TITLE: Method of plasmid DNA production and purification

DEPR:
When the desired OD600 nm of the culture is reached, (as measured off-line in a spectrophotometer), for example, this OD may be of the order of 30-60, the culture is ready for harvest (OD600>60), the fermentation broth is chilled to <10.degree. C. and concentrated to 10 L by standard cross-flow filtration using, for example, a Filtron Centrisette equipped with 100,000 molecular weight cut off or 0.2 polysulphone membranes. The concentrate is then diafiltered against 50 L of a buffer (cell resuspension buffer) such as 50 mM Tris-HCl, 10 mM EDTA, pH 8.0.
At this stage the process stream has an equivalent to 3.5-5.5 kg of biomass (wet weight) containing 1-5 g of plasmid DNA depending on the vector used. The cell slurry obtained at harvest may be stored frozen (<-20.degree. C.) or the cells lysed immediately.

DEPR:
An Amicon CH2 ultrafiltration device was then washed using 0.1M NaOH and equilibrated in 0.75M NaCl, 25 mM KAc 10 mM EDTA at pH 5.5. The eluate was concentrated to approximately 400 ml final volume by ultrafiltration using an SIY-30 Kilodalton molecular weight cut off membrane. The concentrate was removed and decanted into a sterile bottle. Residual plasmid DNA was washed out of the CH2 cartridge with approximately 400 ml of 0.75M NaCl, 25 mM KAc 10 mM EDTA pH 5.5 buffer and pooled with the concentrate. This was stored at 4-10.degree. C.

9. Document ID: US 5916775 A

L8: Entry 9 of 43 File: USPT Jun 29, 1999

US-PAT-NO: 5916775
DOCUMENT-IDENTIFIER: US 5916775 A
TITLE: Method for the purification of DNA
DATE-ISSUED: June 29, 1999

US-CL-CURRENT: 435/91.1; 435/173.7, 435/182, 536/25.4

APPL-NO: 8/ 877874
DATE FILED: June 18, 1997

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	8-157245	June 18, 1996
JP	8-261497	October 2, 1996

IN: Hayashizaki; Yoshihide

AB: Disclosed is a method for collecting DNA by lysing microbial cells, adsorbing released DNA on a carrier and collecting the DNA adsorbed on the carrier, which method comprises the following steps of (1) lysing the microbial cells in the presence of the carrier so that the DNA obtained by lysing cells is adsorbed onto the carrier, separating solutions used for lysing cells and adsorbing DNA from the carrier, and eluting the DNA adsorbed on the carrier with a solution for eluting DNA and collecting eluted DNA, or (2) feeding microbial cells into a column comprising the carrier provided on a membrane filter capable of retaining a solution and permeating the solution when aspirated, lysing the microbial cells in the column so that the DNA obtained by lysing cells is adsorbed onto the carrier, separating solutions used for lysing cells and adsorbing DNA in the previous step from the column by aspiration, and feeding a solution for eluting DNA into the column and aspirating to collect the DNA adsorbed on the carrier. The methods of the present invention enable collection of DNA by the chaotropic ion method employing an apparatus with simpler structure and fewer operations.

L8: Entry 9 of 43 File: USPT Jun 29, 1999

DOCUMENT-IDENTIFIER: US 5916775 A
TITLE: Method for the purification of DNA

BSPR:
For example, Japanese Patent Unexamined Publication No. Hei 4-360686 (JP-A-360686/92) discloses a method for the purification of plasmid DNA and/or cosmid DNA by lysing microbial cells, filtering the resulting lysate with a membrane filter to remove insolubles and subjecting the filtrate to ultrafiltration to remove impurities and concentrate the DNA.

10. Document ID: US 5895646 A

L8: Entry 10 of 43 File: USPT Apr 20, 1999

US-PAT-NO: 5895646
DOCUMENT-IDENTIFIER: US 5895646 A
TITLE: Isolated native primate GM-CSF protein
DATE-ISSUED: April 20, 1999

US-CL-CURRENT: 424/85.1; 514/12, 514/2, 514/8, 530/351, 530/412, 930/145

APPL-NO: 8/ 344809
DATE FILED: November 23, 1994

PARENT-CASE:
This is a continuation of application Ser. No. 08/183,099, filed Jan. 14, 1994, now abandoned, which in turn is a continuation of application Ser. No. 08/023,146, filed Feb. 24, 1993, now abandoned, which in turn is a continuation of application Ser. No. 07/752,250, filed Aug. 28, 1991, now abandoned, which in turn is a continuation of application Ser. No. 07/657,350, filed Feb. 15, 1991, now abandoned, which in turn is a continuation of application Ser. No. 06/652,742, filed Sep. 19, 1984, now abandoned.

IN: Wang; Elizabeth A.

AB: A method for purifying CSF protein is described. The method comprises: precipitating the protein with ammonium sulfate at 80% saturation to form a pellet containing the CSF protein; resuspending the pellet in a buffered solution at a pH in the range of about 6 to about 8; applying the buffered solution containing CSF to a chromatographic column, eluting with the buffered solution containing sodium chloride and collecting the fractions having CSF activity; pooling the active fractions, applying them to a C4 reverse phase column and eluting with a 0 to 90% acetonitrile gradient to collect the active fractions. The purified CSF protein has a specific activity of at least about 1.times.10.sup.7 units per mg of protein and preferably at least about 4.times.10.sup.7 units per mg of protein when assayed using the human bone marrow assay.

L8: Entry 10 of 43 File: USPT Apr 20, 1999

DOCUMENT-IDENTIFIER: US 5895646 A
TITLE: Isolated native primate GM-CSF protein

DEPR:
Plasmid DNA, a cDNA encoding human CSF (as illustrated in FIG. 1) inserted into the eukaryotic expression vector p91023(B) (p91023(B)-CSF) is purified from 2 liters of bacteria by equilibrium density centrifugation in CsCl and ethidium bromide. Details of the construction of vector p91023(B) can be found in copending Ser. No. 628,342. One mg of this DNA was dissolved in 1 ml of 0.1 M Tris, pH 7.3 and added to 600 ml of DME containing 2 mM glutamine, 100 U/ml streptomycin, 100 ug/ml penicillin (P/S) and 0.25 mg/ml DEAE Dextran (Molecular weight 500,000 from Pharmacia). The 600 ml of DNA DEAE Dextran solution is added to the M6 COS cells in the cell factory and incubated at 37.degree. for 12 hours. After the incubation, the cells are rinsed once with 900 ml of SF DME then incubated for 2.5 hours with 600 ml of DME containing 0.1 mM chloroquin, 10% HIFCS, 2 mM glutamine, 100 U/ml penicillin and 100 ug/ml streptomycin. After aspirating the chloroquin containing medium, the cells are rinsed with SF DME and fed 1500 ml of DME with 10% HIFCS. After 30 hours the cells are washed with SF DME, the medium is replaced with 800 ml of SF DME and the transfected cells are allowed to condition the medium for 24 hours at 37.degree. C. The conditioned medium is aspirated and replaced with another 800 ml of SF DME. The cells are allowed to condition this medium for 24 hours then the conditioned medium is collected. As soon as possible after harvesting, the conditioned media sample are concentrated 20 fold by pressurized ultrafiltration using the Amicon 2.5 liter chamber with the YM5 membrane (5,000 MW cutoff).

11. Document ID: US 5837529 A

L8: Entry 11 of 43 File: USPT Nov 17, 1998

US-PAT-NO: 5837529
DOCUMENT-IDENTIFIER: US 5837529 A
TITLE: Method for lysing cells
DATE-ISSUED: November 17, 1998

US-CL-CURRENT: 435/259; 435/306.1

APPL-NO: 8/ 632203
DATE FILED: April 15, 1996

PARENT-CASE:
This application is a continuation of U.S. Ser. No. 08/324,455 filed Oct. 17, 1994, now abandoned.

IN: Wan; Nick C., McNeilly; David S., Christopher; Charles William

AB: This invention relates to a method for lysing cells. The method comprises simultaneously flowing a cell suspension and a lysis solution through a static mixer, wherein the cells exit the static mixer lysed. In another aspect of the present invention, the invention relates to a method for precipitating cell components, protein, and nucleic acids from a cell lysate or other solution containing precipitable material. The method comprises simultaneously flowing a cell lysate or other protein containing solution and a precipitating solution through a static mixer, wherein the lysate or protein solution exits the static mixer with its precipitable components precipitated. In another aspect of the present invention, the invention relates to a method where the two above-mentioned methods above are combined by using static mixers in series.

L8: Entry 11 of 43 File: USPT Nov 17, 1998

DOCUMENT-IDENTIFIER: US 5837529 A
TITLE: Method for lysing cells

DEPR:
This invention is based upon the discovery that static mixers could be used to lyse cells containing plasmids, releasing the plasmids from the cells. The advantage of using such a device is that large volumes of cells can be gently and continuously lysed in-line using the static mixer and that other static mixers could be placed in-line to accomplish other functions such as dilution and precipitation. This method greatly simplifies the process of isolating plasmids from

large volumes of material such that plasmid DNA is not damaged by the process. Previous methods
of plasmid isolation involving caustic lysing and precipitation, which involved expensive and
specialized equipment, were not practical for large scale plasmid purification. The method of the
present invention can be used to lyse any type of cell (i.e., prokaryotic or eukaryotic) for any
purpose related to lysing, such as releasing desired nucleic acids or proteins from target cells
to be subsequently purified. In a preferred embodiment, the method of the present invention is
used to lyse host cells containing plasmids to release plasmids.

12. Document ID: US 5827706 A

L8: Entry 12 of 43

File: USPT

Oct 27, 1998

US-PAT-NO: 5827706
DOCUMENT-IDENTIFIER: US 5827706 A
TITLE: Cyclosporin synthetase
DATE-ISSUED: October 27, 1998

US-CL-CURRENT: 435/183; 435/320.1, 435/325, 536/23.2

APPL-NO: 8/471119
DATE FILED: June 6, 1995

PARENT-CASE:
This is a continuation of application Ser. No. 08/263,960, filed Jun. 20, 1994 and now abandoned,
which is a continuation of application Ser. No. 08/090,552, filed Jul. 9, 1993 and now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
AT	1403/92	July 9, 1992
AT	437/93	March 8, 1993
CH	01310/93	April 29, 1993
CH	01375/93	May 4, 1993

IN: Leitner; Ernst, Schneider; Elisabeth, Schoergendorfer; Kurt, Weber; Gerhard

AB: The nucleotide sequence which codes for cyclosporin synthetase and similar
enzymes and recombinant vectors containing the sequence. The vectors are used in methods for
the production of cyclosporin and cyclosporin derivatives.

L8: Entry 12 of 43

File: USPT

Oct 27, 1998

DOCUMENT-IDENTIFIER: US 5827706 A

TITLE: Cyclosporin synthetase

DEPR:
A plasmid containing a 2.7 kb EcoRI-HindIII fragment from Example 18a cloned into pUC18 is
linearised with HindIII. 1 ng of the plasmid DNA is amplified with the oligonucleotides described
above (Sambrook et al., 1989): 30 cycles: 1 minutes 30 sec 94.degree. C.; 2 min 30 sec 50.degree.
C.; 6 min 72.degree. C. A 2.1 kb DNA is produced. After chloroform extraction, this DNA is
purified by ultrafiltration (Ultrafree MC 100 000; Millipore) and cleaved in the appropriate
buffer with the enzymes ClaI and BamHI. 50 ng of this DNA are ligated with 50 ng of BamHI and
ClaI cleaved DNA of the plasmid pGEM7Zf (Promega). The newly produced plasmid is cleaved with
ClaI and XbaI and ligated with a ClaI-XbaI restriction fragment 1.76 kb in size from the plasmid
pCSN44 (Staben et al., 1989). A restriction map of this plasmid (pSIM10) is reproduced in FIG. 3.

13. Document ID: US 5795568 A

L8: Entry 13 of 43

File: USPT

Aug 18, 1998

US-PAT-NO: 5795568
DOCUMENT-IDENTIFIER: US 5795568 A
TITLE: Method of treating infectious disease with GM-CSF
DATE-ISSUED: August 18, 1998

US-CL-CURRENT: 424/85.1; 424/184.1, 424/198.1, 514/12, 514/2, 514/8, 514/885

APPL-NO: 8/469530
DATE FILED: June 6, 1995

PARENT-CASE:
This is a division of application Ser. No. 08/344,806, filed Nov. 23, 1994, which in turn is a
continuation of application Ser. No. 08/183,099, filed Jan. 14, 1994, now abandoned, which in
turn is a continuation of application Ser. No. 08/023,146, filed Feb. 24, 1993, now abandoned,
which in turn is a continuation of application Ser. No. 07/752,250, filed Aug. 28, 1991, now
abandoned, which in turn is a continuation of application Ser. No. 07/657,350, filed Feb. 15,
1991, now abandoned, which in turn is a continuation of application Ser. No. 06/652,742, filed
Sep. 19, 1994, now abandoned.

IN: Wang; Elizabeth A.

AB: A method for purifying CSF protein is described. The method comprises:
precipitating the protein with ammonium sulfate at 80% saturation to form a pellet
containing the CSF protein; resuspending the pellet in a buffered solution at a pH in the
range of about 6 to about 8; applying the buffered solution containing CSF to a
chromatographic columns eluting with the buffered solution containing sodium chloride and
collecting the fractions having CSF activity; pooling the active fractions, applying them to
a C4 reverse phase column and eluting with a 0 to 90% acetonitrile

gradient to collect the
active fractions. The purified CSF protein has a specific activity of at least about
1.times.10.sup.7 units per mg of protein and preferably at least about 4.times.10.sup.7
units per mg of protein when assayed using the human bone marrow assay.

L8: Entry 13 of 43 File: USPT Aug 18, 1998

DOCUMENT-IDENTIFIER: US 5795568 A
TITLE: Method of treating infectious disease with GM-CSF

DEPR:
Plasmid DNA, a CDNA encoding human CSF (as illustrated in FIG. 1) inserted into the eukaryotic
expression vector p91023(B) (p091023(B)-CSF) is purified from 2 liters of bacteria by equilibrium
density centrifugation in CsCl and ethidium bromide. Details of the construction of vector
p91023(B) can be found in copending Ser. No. 628,342. One mg of this DNA was dissolved in 1 ml of
0.1M Tris, pH 7.3 and added to 600 ml of DME containing 2 mM glutamine, 100 U/mi streptomycin,
100 ug/ml penicillin (P/S) and 0.25 mg/ml DEAE Dextran (Molecular weight 500,000 from Pharmacia).
The 600 ml of DNA DEAE Dextran solution is added to the M6 COS cells in the cell factory and
incubated at 37.degree. for 12 hours. After the incubation, the cells are rinsed once with 900 ml
of SF DME then incubated for 2.5 hours with 600 ml of DME containing 0.1 mM chloroquin, 10%
HIFCS. 2 mM glutamine, 100 U/ml penicillin and 100 ug/ml streptomycin. After aspirating the
chloroquin containing medium, the cells are rinsed with SF DME and fed 1500 ml of DME with 10%
HIFCS. After 30 hours the cells are washed with SF DME, the medium is replaced with 800 ml of SF
DME and the transfected cells are allowed to condition the medium for 24 hours at 37.degree. C.
The conditioned medium is aspirated and replaced with another 800 mil of SF DME. The cells are
allowed ton condition this medium or 24 hours then the conditioned medium is. collected. As soon
as possible after harvesting, the conditioned, media sample are concentrated 20 fold by
pressurized ultrafiltration using the Amicon 2.5 liter chamber with the YM5 membrane (5,000 MW
cutoff).

14. Document ID: US 5789224 A

L8: Entry 14 of 43 File: USPT Aug 4, 1998

US-PAT-NO: 5789224
DOCUMENT-IDENTIFIER: US 5789224 A
TITLE: Recombinant expression vectors and purification methods for thermus thermophilus DNA
polymerase
DATE-ISSUED: August 4, 1998

US-CL-CURRENT: 435/194

APPL-NO: 8/ 459383

DATE FILED: June 2, 1995

PARENT-CASE:
CROSS-REFERENCE This application is a divisional, of application Ser. No. 08/384,490 filed Feb.
6, 1995, now U.S. Pat. No. 5,618,711, which is a continuation of Ser. No. 08/148,133, filed Nov.
2, 1993 now abandoned, which is a continuation of U.S. Ser. No. 07/880,478, filed May 6, 1992,
now abandoned, which is a continuation of U.S. Ser. No. 07/455,967, filed Dec. 22, 1989, now
abandoned, which is a continuation-in-part of U.S. Ser. No. 07/143,441, filed Jan. 12, 1988, now
abandoned, which is a continuation-in-part of U.S. Ser. No. 07/063,509, filed Jun. 17, 1987,
which issued as U.S. Pat. No. 4,889,818, which is a continuation-in-part of U.S. Ser. No.
06/899,241, filed Aug. 22, 1986, now abandoned.

IN: Gelfand; David H., Lawyer; Frances C., Stoffel; Susanne

AB: Recombinant DNA sequences encoding the DNA polymerase activity of Thermus
thermophilus can be used to construct recombinant vectors and transformed host cells for
production of the activity. T. thermophilus DNA polymerase is an .about.94 kDa protein
especially useful in the DNA amplification procedure known as the polymerase chain reaction.

L8: Entry 14 of 43 File: USPT Aug 4, 1998

DOCUMENT-IDENTIFIER: US 5789224 A
TITLE: Recombinant expression vectors and purification methods for thermus thermophilus DNA
polymerase

BSPR:
In the fifth stage, the fraction collected in the fourth step is concentrated and diafiltered
against Affigel blue buffer. The precipitate formed is removed by centrifugation, and the
supernatant is applied to an Affigel-blue column equilibrated with 0.1M KCl. The column is then
washed with 0.1M KCl and the enzyme eluted with a linear gradient of a buffer such as 0.1 to 0.5M
KCl. Fractions with thermostable enzyme activity are then tested for contaminating
deoxyribonucleases (endo- and exonucleases) using any suitable procedure. For example, the
endonuclease activity may be determined electrophoretically from the change in molecular weight
of phage .lambda. DNA or supercoiled plasmid DNA after incubation with an excess of DNA
polymerase. Similarly, exonuclease activity may be determined electrophoretically from the change
in molecular weight of DNA after treatment with a restriction enzyme that cleaves at several
sites. The fractions determined to have no deoxyribonuclease activity (peak activity of
polymerase elutes at 0.28 to 0.455M KCl) are pooled and dialyzed against CM-Trisacryl buffer. The
precipitate formed is removed by centrifugation.

15. Document ID: US 5714374 A

L8: Entry 15 of 43

File: USPT

Feb 3, 1998

US-PAT-NO: 5714374
DOCUMENT-IDENTIFIER: US 5714374 A
TITLE: Chimeric rhinoviruses
DATE-ISSUED: February 3, 1998

US-CL-CURRENT: 435/235.1; 424/93.6

APPL-NO: 8/ 406347
DATE FILED: March 17, 1995

PARENT-CASE:
This application is a Continuation-in-part of U.S. Ser. No. 08/304,635 filed Sep. 12, 1994, now
U.S. Pat. No. 5,541,100, which is a continuation of Ser. No. 08/041,790, filed Apr. 1, 1993, now
abandoned, which in turn is a continuation of Ser. No. 07/582,335, filed Sep. 12, 1990, now
abandoned.

IN: Arnold; Edward V., Arnold; Gail Ferstandig

AB: Various novel recombinant chimeric human rhinoviruses are disclosed, including
viruses comprising human rhinovirus 14 into which chimeric regions derived from influenza
HA, poliovirus and HIV-1 have been incorporated. Chimeric human rhinoviruses are
particularly advantageous as they are only mildly pathogenic, have numerous potential
serotypes and can elicit significant mucosal and serum immunological response. Design
considerations, methods, and examples are described. The chimeric rhinoviruses can be used
as vaccines and for a variety of other immunotechnological applications including passive
immunization, immunodiagnostic testing and antigenicity and immunogenicity studies.

L8: Entry 15 of 43

File: USPT

Feb 3, 1998

DOCUMENT-IDENTIFIER: US 5714374 A
TITLE: Chimeric rhinoviruses

DEPR:
The chimeric rhinovirus to be used in this invention can be generated from a stable source of
plasmid DNA, or later from seed stocks of the chimeric HRV. Using the techniques described, the
recombinant chimeric human rhinoviruses generated will have the exact desired sequence content
and length of amino acids and will not have any undesired amino acids that could result from
using a restrictive mutagenesis cassette. When the chimeric HRV is to be produced in large
amounts, large numbers of cells can be accommodated either in suspension cultures and/or on
carriers such as microcarrier beads. Propagations can be performed in transformed human cells,
such as the H1-HeLa cells used in this work, or preferably in non-transformed human cells, such
as human diploid fibroblast cells (WI-38, MRC-5, etc.). Virus can be obtained in purified form
from infected cells following cell lysis. Examples of purification steps include standard
differential centrifugation techniques, concentration by ultrafiltration or pressure dialysis, or
concentration by precipitation.

16. Document ID: US 5707812 A

L8: Entry 16 of 43

File: USPT

Jan 13, 1998

US-PAT-NO: 5707812
DOCUMENT-IDENTIFIER: US 5707812 A
TITLE: Purification of plasmid DNA during column chromatography
DATE-ISSUED: January 13, 1998

US-CL-CURRENT: 435/6; 435/252.3, 435/320.1, 536/23.1

APPL-NO: 8/ 692590
DATE FILED: August 6, 1996

IN: Horn; Nancy, Budahazi; Greg, Marquet; Magda

AB: A method for purifying plasmid DNA during column chromatography is provided. A
short chain polymeric alcohol, preferably polyethylene glycol, or another DNA condensation
agent, is added to a DNA sample prior to column chromatography. The short chain polymeric
alcohol or condensation agent promotes improved isolation of plasmid DNA and may be used for
large scale purification, particularly for manufacturing plasmid DNA as a biopharmaceutical.

L8: Entry 16 of 43

File: USPT

Jan 13, 1998

DOCUMENT-IDENTIFIER: US 5707812 A
TITLE: Purification of plasmid DNA during column chromatography

BSPR:
The use of short chain polymeric alcohols, like polyethylene glycol, and other condensation
agents that cause plasmid DNA to act homogeneously for purposes of purification is not limited to
ion exchange chromatography. It extends to other chromatographic methods, including size
exclusion chromatography, chromatofocusing, affinity chromatography, hydrophobic interaction
chromatography, and reversed phase chromatography. Indeed, this use extends broadly to other
purification methods, e.g., diafiltration, ultrafiltration, and filtration generally, in which
the isolation of plasmid DNA from RNA, proteins and other contaminants is facilitated by causing
various plasmid DNA species to act as a class.

17. Document ID: US 5695964 A

L8: Entry 17 of 43

File: USPT

Dec 9, 1997

US-PAT-NO: 5695964
DOCUMENT-IDENTIFIER: US 5695964 A

TITLE: Recombinant DNA vectors, including plasmids, and host cells for production of truncated
thrombomodulin
DATE-ISSUED: December 9, 1997

US-CL-CURRENT: 435/69.6; 435/243, 435/320.1, 435/325, 435/358

APPL-NO: 8/ 587389
DATE FILED: January 17, 1996

PARENT-CASE:
This is a Division of application Ser. No. 08/307,444 filed Sep. 19, 1994, now issued as U.S.
Pat. No. 5,516,659, which in turn is a Continuation of application Ser. No. 07/835,436 filed Mar.
27, 1992, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY
APPL-NO
APPL-DATE
JP
Hei 2-168766
June 27, 1990

IN: Nii; Atsushi, Morishita; Hideaki, Uemura; Akio, Mochida; Ei

AB: This invention relates to novel vectors and host cells containing nucleic acids
coding for a polypeptide having thrombin binding ability, anticoagulant activity and
thrombolytic activity. The polypeptide can be efficiently produced in large quantities by
means of genetic recombination techniques using the vectors and host cells of the present
invention. Since the polypeptide exhibits anticoagulant activity and thrombolytic activity
without generating side effects such as bleeding tendencies, it can be applied effectively
to the prevention and treatment of hypercoagulability-related diseases.

L8: Entry 17 of 43
File: USPT
Dec 9, 1997

DOCUMENT-IDENTIFIER: US 5695964 A
TITLE: Recombinant DNA vectors, including plasmids, and host cells for production of truncated
thrombomodulin

BSPR:
Each of the plasmids pKCR-TM-Ala and pKCR-TM-Val prepared in Example 1 was transfected into COS-7
cells (ATCC No. CRL1651) by means of DEAE dextran method to express recombinant thrombomodulin.
That is, semiconfluent COS-7 cells prepared in advance were transfected with the plasmid DNA at a
ratio of about 1 .mu.g DNA per about 2.times.10.sup.5 cells in accordance with the method of
Lauren et al. (Lauren, M., Proc. Natl. Acad. Sci. USA, Vol.78, p.7575, 1981). The thus treated
cells were cultured for 3 days using Dulbecco's modified Eagle's medium (to be referred to as
"D-ME medium" hereinafter) which has been supplemented with 0.01% albumin, followed by recovering
of culture supernatant to obtain a crude recombinant human urine thrombomodulin solution.
Transfection was carried out in the same manner and a 10 liter portion of the resulting culture
filtrate was subjected to desalting and concentration making use of an ultrafiltration membrane
of 30,000-molecular-weight cutoff.

18. Document ID: US 5624833 A

L8: Entry 18 of 43
File: USPT
Apr 29, 1997

US-PAT-NO: 5624833
DOCUMENT-IDENTIFIER: US 5624833 A
TITLE: Purified thermostable nucleic acid polymerase enzyme from Thermotoga maritima
DATE-ISSUED: April 29, 1997

US-CL-CURRENT: 435/194

APPL-NO: 8/ 475231
DATE FILED: June 7, 1995

PARENT-CASE:
This application is a divisional of application Ser. No. 07/971,819 filed Feb. 3, 1993, now U.S.
Pat. No. 5,420,029, which is a continuation-in-part of Ser. No. 07/567,244, filed Aug. 13, 1990,
now U.S. Pat. No. 5,374,553, which is a continuation-in-part of Ser. No. 07/143,441, filed Jan.
12, 1988, now abandoned, which is a continuation-in-part of Ser. No. 07/063,509, filed Jun. 17,
1987, now U.S. Pat. No. 4,889,818, which is a continuation-in-part of Ser. No. 06/899,241, filed
Aug. 22, 1986, now abandoned.

IN: Gelfand; David H., Lawyer; Frances C., Stoffel; Susanne

AB: A purified thermostable enzyme is derived from the eubacterium Thermotoga
maritima. The enzyme has a molecular weight as determined by gel electrophoresis of about 97
kilodaltons and DNA polymerase I activity. The enzyme can be produced from native or
recombinant host cells and can be used with primers and nucleoside triphosphates in a
temperature-cycling chain reaction where at least one nucleic acid sequence is amplified in
quantity from an existing sequence.

L8: Entry 18 of 43
File: USPT
Apr 29, 1997

DOCUMENT-IDENTIFIER: US 5624833 A
TITLE: Purified thermostable nucleic acid polymerase enzyme from Thermotoga maritima

BSPR:
In the fifth stage, the fraction collected in the fourth stage is diluted with affigel-blue
buffer without KCl and applied to an affigel-blue column equilibrated in 25 mM Tris-Cl (pH 7.5),
0.1 mM EDTA, 0.2% Tween 20, 0.5 mM DTT, and 0.15M KCl. The column is washed with the same buffer
and eluted with a linear gradient of 0.15M to 0.7M KCl in the same buffer. The peak activity
fractions were found at the 0.3M to 0.55M KCl section of the gradient. These fractions of peak
activity are then tested for contaminating deoxyribonucleases (endonucleases and exonucleases)
using any suitable procedure. As an example, endonuclease activity may be determined
electrophoretically from the change in molecular weight of phage .lambda.

DNA or supercoiled
plasmid DNA after incubation with an excess of DNA polymerase. Similarly, exonuclease activity
may be determined electrophoretically from the change in molecular weight of restriction enzyme
digested DNA after incubation with an excess of DNA polymerase. The fractions that have no
deoxyribonuclease activity are pooled and diafiltered into phosphocellulose buffer containing 50
mM KCl.

19. Document ID: US 5618711 A

L8: Entry 19 of 43
File: USPT
Apr 8, 1997

US-PAT-NO: 5618711
DOCUMENT-IDENTIFIER: US 5618711 A
TITLE: Recombinant expression vectors and purification methods for Thermus thermophilus DNA
polymerase
DATE-ISSUED: April 8, 1997

US-CL-CURRENT: 435/194; 435/252.33, 435/320.1, 536/23.2

APPL-NO: 8/ 384490
DATE FILED: February 6, 1995

PARENT-CASE:
CROSS-REFERENCE This application is a continuation of U.S. patent application Ser. No.
08/148,133, filed Nov. 02, 1993, now abandoned, which is a continuation of U.S. patent
application Ser. No. 07/880,478, filed May 6, 1992, which is a continuation of U.S. patent
application Ser. No. 07/455,967, filed Dec. 22, 1989, now abandoned, which is a
continuation-in-part of U.S. patent application Ser. No. 07/143,441, filed Jan. 12, 1988, now
abandoned, which is a continuation-in-part of U.S. patent application Ser. No. 07/063,509, filed
Jun. 17, 1987, which issued as U.S. Pat. No. 4,889,818, which is a continuation-in-part of U.S.
patent application Ser. No. 06/899,241, filed Aug. 22, 1986, now abandoned.

IN: Gelfand; David H., Lawyer; Frances C., Stoffel; Susanne

AB: Recombinant DNA sequences encoding the DNA polymerase activity of Thermus
thermophilus can be used to construct recombinant vectors and transformed host cells for
production of the activity. T. thermophilus DNA polymerase is an .about.94 kDa protein
especially useful in the DNA amplification procedure known as the polymerase chain reaction.

L8: Entry 19 of 43
File: USPT
Apr 8, 1997

DOCUMENT-IDENTIFIER: US 5618711 A
TITLE: Recombinant expression vectors and purification methods for Thermus thermophilus DNA
polymerase

DEPR:
In the fifth stage, the fraction collected in the fourth step is concentrated and diafiltered
against Affigel blue buffer. The precipitate formed is removed by centrifugation, and the
supernatant is applied to an Affigel-blue column equilibrated with 0.1M KCl. The column is then
washed with 0.1M KCl and the enzyme eluted with a linear gradient of a buffer such as 0.1 to 0.5M
KCl. Fractions with thermostable enzyme activity are then tested for contaminating
deoxyribonucleases (endo- and exonucleases) using any suitable procedure. For example, the
endonuclease activity may be determined electrophoretically from the change in molecular weight
of phage .lambda. DNA or supercoiled plasmid DNA after incubation with an excess of DNA
polymerase. Similarly, exonuclease activity may be determined electrophoretically from the change
in molecular weight of DNA after treatment with a restriction enzyme that cleaves at several
sites. The fractions determined to have no deoxyribonuclease activity (peak activity of
polymerase elutes at 0.28 to 0.455M KCl) are pooled and dialyzed against CM-Trisacryl buffer. The
precipitate formed is removed by centrifugation.

20. Document ID: US 5616476 A

L8: Entry 20 of 43
File: USPT
Apr 1, 1997

US-PAT-NO: 5616476
DOCUMENT-IDENTIFIER: US 5616476 A
TITLE: Synthetic isohirudins with improved stability
DATE-ISSUED: April 1, 1997

US-CL-CURRENT: 435/69.1; 435/252.3, 435/320.1, 514/12, 530/326, 530/412, 530/416, 530/418,
536/22.1, 536/23.5

APPL-NO: 8/ 452829
DATE FILED: May 30, 1995

PARENT-CASE:
This application is a continuation of application Ser. No. 08/155,753, filed Nov. 22, 1993, now
abandoned, which is division of application Ser. No. 08/099,053, filed Jul. 29, 1993, now U.S.
Pat. No. 5,316,947, which is a division of Ser. No. 07/985,110, filed Dec. 3, 1992, now U.S. Pat.
No. 5,286,714.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	41 40 381.9	December 7, 1991

IN: Crause; Peter, Habermann; Paul, Tripier; Dominique, Ulmer; Wolfgang, Schmid;
Gerhard

AB: The invention relates to novel synthetic isohirudins which have improved
stability owing to exchange in the region of the Asp-Gly motif. This

results, on the one
hand, in an increase in the yield during workup and, on the other hand, in making possible
pharmaceutical formulation as directly injectable solution ready for use.

L8: Entry 20 of 43
File: USPT
Apr 1, 1997

DOCUMENT-IDENTIFIER: US 5616476 A
TITLE: Synthetic isohirudins with improved stability

DEPR:
The cloning vector 7 described in European Patent Application EP-A 324 712 is opened with BamHI
and HindIII and in each case ligated to the BamHI/HindIII fragment which has been isolated from
the plasmid pSCH13 or pSCH93 and which comprises amino acids of the carboxyl-terminal part of the
hirudin sequence which are missing from the cloning vector in each case. The plasmids p713 and
p793 are produced and are characterized by restriction analysis. Subsequently, the EcoRI/HindIII
fragment is isolated from correct DNA of these plasmids, and the protruding ends are filled in a
Klenow polymerase reaction. The fragments prepared in this way are ligated in each case to the
blunt-ended vector fragment from the plasmid yEP13 as described in Example 1 of European Patent
Application EP-A 324 712. The plasmids pHABVar131 and pHABVar132 which differ only with regard to
the orientation of the inserted fragment and which code for a hirudin derivative which has the
amino acids Leu.sup.1, Glu.sup.33, Gln.sup.52, Glu.sup.53 and Glu.sup.55, and the plasmids
pHABVar931 and pHABVar932 which likewise differ only in the orientation of the inserted fragment
and which code for a hirudin derivative which has the amino acids Leu.sup.1, Glu.sup.33,
Gln.sup.52, Glu.sup.53 and Ala.sup.54 are produced. The plasmids are, by way of example,
transformed into the yeast strains described in the Application. Expression and purification of
the hirudin derivatives can be carried out by the procedure described therein. It is known that
it is possible in the purification to dispense with centrifugation and subsequent adsorption
chromatography when, for example, the Millipore Pellicon ultrafiltration system is used. The
methods used here are described for the laboratory scale. For cultures on the cubic meter scale,
other fermentation times, culture conditions and steps in the workup may be necessary. This is
known to the person skilled in the art.

21. Document ID: US 5561064 A

L8: Entry 21 of 43
File: USPT
Oct 1, 1996

US-PAT-NO: 5561064
DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA
DATE-ISSUED: October 1, 1996

US-CL-CURRENT: 435/320.1; 435/259, 435/91.1

APPL-NO: 8/ 192151
DATE FILED: February 1, 1994

IN: Marquet; Magda, Horn; Nancy, Meek; Jennifer, Budahazi; Gregg

AB: The invention relates to a method for producing plasmid DNA, comprising the steps
of: (a) lysing cells containing the plasmid DNA to obtain a lysate; (b) treating the lysate
by a means for removing insoluble material to obtain a solute; and (c) applying the solute
to differential PEG precipitations and chromatography to purify the plasmid DNA. In other
embodiments of the invention, the plasmid DNA is produced with GRAS reagents; the plasmid
DNA is produced in the absence of enzymes; the plasmid DNA is produced in the absence of
organic extractants; the plasmid DNA is produced in the absence of mutagens; the lysing,
treating and applying steps are scalable to result in the large scale manufacture of the
plasmid DNA; and the lysing, treating and applying steps result in the generation of
pharmaceutical grade material.

L8: Entry 21 of 43
File: USPT
Oct 1, 1996

DOCUMENT-IDENTIFIER: US 5561064 A
TITLE: Production of pharmaceutical-grade plasmid DNA

DEPR:
(At this point in the experiment, it was decided to concentrate the plasmid DNA filtrate by a
2-propanol precipitation in order to load the material on a Pharmacia S-1000 column (Pharmacia,
Piscataway, N.J.) as soon as possible to determine the yield and spectrum of impurities. In
practice, the plasmid DNA would be concentrated by anion exchange, ultrafiltration, or a second
PEG-8000 precipitation.)

22. Document:ID: US 5541100 A

L8: Entry 22 of 43
File: USPT
Jul 30, 1996

US-PAT-NO: 5541100
DOCUMENT-IDENTIFIER: US 5541100 A
TITLE: Chimeric rhinoviruses
DATE-ISSUED: July 30, 1996

US-CL-CURRENT: 435/235.1; 424/93.6

APPL-NO: 8/ 304635
DATE FILED: September 12, 1994

PARENT-CASE:
This application is a continuation of U.S. Ser. No. 08/041,790, filed Apr. 1, 1993, abandoned,
which is a continuation of application Ser. No. 07/582,335 filed on Sep. 12, 1990, now abandoned.

IN: Arnold; Edward V., Arnold; Gail F.

AB: Recombinant chimeric human rhinovirus and method for stimulation of a specific immune response. Design considerations, methods, and examples are described. Chimeric rhinoviruses can be used as vaccines and for a variety of other immunotechnological applications.

L8: Entry 22 of 43

File: USPT

Jul 30, 1996

DOCUMENT-IDENTIFIER: US 5541100 A
TITLE: Chimeric rhinoviruses

BSPR:
The chimeric rhinovirus to be used in this invention can be generated from a stable source of plasmid DNA, or later from seed stocks of the chimeric HRV. Using the techniques described, the recombinant chimeric human rhinoviruses generated will have the exact desired sequence content and length of amino acids and will not have any undesired amino acids that could result from using a restrictive mutagenesis cassette. When the chimeric HRV is to be produced in large amounts, large numbers of cells can be accommodated either in suspension cultures and/or on carriers such as microcarrier beads. Propagations can be performed in transformed human cells, such as the H1-HeLa cells used in this work, or preferably in non-transformed human cells, such as human diploid fibroblast cells (WI-38, MRC-5, etc.). Virus can be obtained in purified form from infected cells following cell lysis. Examples of purification steps include standard differential centrifugation techniques, concentration by ultrafiltration or pressure dialysis, or concentration by precipitation.

23. Document ID: US 5516659 A

L8: Entry 23 of 43

File: USPT

May 14, 1996

US-PAT-NO: 5516659
DOCUMENT-IDENTIFIER: US 5516659 A
TITLE: Truncated thrombomodulin, recombinant production thereof, and therapeutic agent
DATE-ISSUED: May 14, 1996

US-CL-CURRENT: 435/69.6; 514/2, 514/8, 530/350, 530/395, 536/23.5

APPL-NO: 8/ 307444
DATE FILED: September 19, 1994

PARENT-CASE:
This is a continuation of application Ser. No. 07/835,436 filed Mar. 27, 1992, now abandoned.

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
JP	2-168766	June 27, 1990

IN: Nii; Aïsushi, Morishita; Hideaki, Uemura; Akio, Mochida; Ei

AB: This invention relates to a novel polypeptide which is obtained by means of genetic recombination DNA techniques and has thrombin binding ability, anticoagulant activity and thrombolytic activity. The polypeptide of the present invention can be produced in a large quantity and efficiently by means of genetic recombination techniques. Since the polypeptide of the present invention exhibits anticoagulant activity and thrombolytic activity without generating side effects such as bleeding tendency, it can be applied effectively to the prevention and treatment of hypercoagulability-related diseases.

L8: Entry 23 of 43

File: USPT

May 14, 1996

DOCUMENT-IDENTIFIER: US 5516659 A
TITLE: Truncated thrombomodulin, recombinant production thereof, and therapeutic agent

BSPR:
Each of the plasmids pKCR-TM-Ala and pKCR-TM-Val prepared in Example 1 was transfected into COS-7 cells (ATCC No. CRL1651) by means of DEAE dextran method to express recombinant thrombomodulin.
That is, semiconfluent COS-7-cells prepared in advance were transfected with the plasmid DNA at a ratio of about 1 .mu.g DNA per about 2.times.10.sup.5 cells in accordance with the method of Lauren et al. (Lauren, M., Proc. Natl. Acad. Sci., USA, Vol. 78, p. 7575, 1981). The thus treated cells were cultured for 3 days using Dulbecco's modified Eagle's medium (to be referred to as "D-ME medium" hereinafter) which has been supplemented with 0.01% albumin, followed by recovering of culture supernatant to obtain a crude recombinant human urine thrombomodulin solution.
Transfection was carried out in the same manner and a 10 liter portion of the resulting culture filtrate was subjected to desalting and concentration making use of an ultrafiltration membrane of 30,000-molecular-weight cutoff.

24. Document ID: US 5508261 A

L8: Entry 24 of 43

File: USPT

Apr 16, 1996

US-PAT-NO: 5508261
DOCUMENT-IDENTIFIER: US 5508261 A
TITLE: Analogs of glycoprotein hormones having altered receptor binding specificity and activity and methods for preparing and using same
DATE-ISSUED: April 16, 1996

US-CL-CURRENT: 514/8; 530/397, 530/398

APPL-NO: 8/ 425673
DATE FILED: April 18, 1995

PARENT-CASE:
This is a continuation application of parent application Ser. No.: 08/184,408 filed on 21 Jan. 1994, now abandoned, which is a continuation-in-part application of parent application Ser. No. 08/108,845, filed on 18 Aug. 1993, now abandoned, which is a continuation application of patent application Ser. No. 07/717,151, filed 18 Jun. 1991, now abandoned.

IN: Moyle; William R., Campbell; Robert K., Macdonald; Gordon J., Han; Yi, Wang; Yanhong

AB: The present invention pertains to an alpha, beta-heterodimeric polypeptide having binding affinity to vertebrate luteinizing hormone (LH) receptors and vertebrate follicle stimulating hormone (FSH) receptors comprising a glycoprotein hormone alpha-subunit polypeptide and a non-naturally occurring beta-subunit polypeptide, wherein the beta-subunit polypeptide is a chain of amino acids comprising the following four joined subsequences:, (a) a first subsequence homologous to the amino acid sequence of residues 1-93 of the beta-subunit selected from the group consisting of human chorionic gonadotrophin (hCG), vertebrate luteinizing hormone (LH), vertebrate follicle stimulating hormone (FSH), and vertebrate thyroid stimulating hormone (TSH);, (b) a second subsequence homo logous to the amino acid sequence of residues 94-97 of the beta-subunit selected from the group consisting of human chorionic gonadotrophin (hCG) and vertebrate luteinizing hormone (LH);, (c) a third subsequence homologous to the amino acid sequence of residues 98-100 of the beta-subunit selected from the group consisting of human chorionic gonadotrophin (hCG), vertebrate luteinizing hormone (LH), vertebrate follicle stimulating hormone (FSH), and vertebrate thyroid stimulating hormone (TSH); and, (d) a fourth subsequence homologous to the amino acid sequence of residues 101-110 of the beta-subunit of vertebrate follicle stimulating hormone.

L8: Entry 24 of 43

File: USPT

Apr 16, 1996

DOCUMENT-IDENTIFIER: US 5508261 A

TITLE: Analogs of glycoprotein hormones having altered receptor binding specificity and activity and methods for preparing and using same

DEPR:
An aliquot of the ligation mixture was taken and used to transform DH5-alpha strain E. coli. (obtained from Bethesda Research Laboratories, Gaithersburg, Md.). Plasmid DNAs from ampicillin-resistant DH5-alpha clones were screened by digestion with BglII (which is unique to vectors containing the cassette) and EcoRI (which cuts in the vector). Positive clones were identified by the presence of two fragments (approximately 0.8 Kbp and 2.9 Kbp). The sequence in the coding region of one of these plasmids, which lacked most of the beta-subunit cDNA due to excision of the PvuII fragment, was confirmed by dideoxysequencing as described (10). The remainder of the beta-subunit cDNA (encoding hCG.beta. amino acids 1-87) was restored by ligation of the 2.3 Kbp PvuI-PvuII fragment of this vector and the 2.9 Kbp PvuI-PvuII fragment from pSVL-hCG-beta'. The ligation mixture was used to transform DH5-alpha strain E. coli. and ampicillin resistant clones were obtained. Miniprep plasmid DNA from these clones were digested with EcoRI and BglII, and DNA from positive clones exhibited fragments of approximately 2.5 Kbp and 2.9 Kbp. After the DNA was subjected to a dideoxy sequencing procedure to confirm that it encoded "GT" (Table 1), the plasmid DNA was then cotransfected into COS-7 cells (obtained from the American Type Culture Collection) along with pSVL-hCG-alpha, a pSVL-based plasmid encoding the human glycoprotein hormone alpha-subunit (10, 21), using a DEAE-dextran procedure (10).
Beginning in 1-2 days and for a few days thereafter, the COS-7 cells produced significant amounts of the free subunits and the heterodimer. These were present in the culture media and heterodimer was detected using sandwich immunoassays employing monoclonal antibodies All3 and B105 (10). The protein was concentrated by ultrafiltration and monitored for its abilities to bind to LH and FSH receptors by radioligand receptor assays using .sup.125 I-hCG and .sup.125 I-hFSH as tracers and rat ovarian corpora lutea and bovine testes as sources of LH and FSH receptors as described (10).

DEPR:
We have found that an alpha, beta-heterodimer composed of the alpha-subunit of hCG and an hCG/hFSH beta-subunit chimera termed "G" having the amino acid sequence illustrated in Table 1 has high affinity for LH and FSH receptors as shown by its ability to compete with radiolabeled hCG and/or hFSH for binding to these receptors (Table 3, FIGS. 1 and 2). This analog can be prepared in a variety of methods well-known to one versed in the art of molecular biology, one of which is described here. The cDNA for analog "GT' was digested with BglII and SstI and the 5.2-5.3 Kbp fragment was ligated with the oligonucleotides: ##STR4## using standard methods (23, 24). The ligation mixture was used to transform competent DH5-alpha strain E. coli. (23, 24).
Transformed cells were selected by their abilities to grow on agar plates containing amphicillin.
Ampicillin resistant colonies were chosen and plasmid minipreparations were made by the boiling lysis method (23, 24). The plasmid DNA was then tested for the presence of HindIII-ApaI endonuclease restriction sites. Plasmid DNA having the desired sequences was cleaved into three fragments (approximately 0.8 Kbp, 1.1 Kbp, and 3.4 Kbp). After the DNA was subjected to a dideoxy sequencing procedure to confirm that it encoded "G" (Table 1), the plasmid DNA was then cotransfected into COS-7 cells (obtained from the American Type Culture Collection) along with pSVL-hCG-alpha, a pSVL-based plasmid encoding the alpha-subunit (10, 21), using a DEAE-dextran procedure (10, 21, 23, 24). Beginning in 1-2 days and for a few days thereafter, the COS-7 cells produced significant amounts of the free subunits and the heterodimer. These were present in the culture media and heterodimer was detected using sandwich immunoassays employing monoclonal antibodies A113 and B105 (10). The protein was concentrated by ultrafiltration and monitored for its abilities to bind to LH and FSH receptors by radioligand receptor assays using .sup.125 I-hCG and .sup.125 I-hFSH as tracers and rat ovarian corpora lutea and bovine testes as sources of LH and FSH receptors as described (10).

DEPR:
The ligation mixture was used to transform DH5A E. coli. and miniprep

plasmid DNA obtained from
ampicillin resistant colonies was screened for the presence of an approximately 0.6Kbp fragment
released by digestion with AccI. After DNA sequencing was performed to confirm that the construct
encoded the desired sequence, it was cut with PvuII and ligated with the 1.6 Kbp fragment of
pSVL-hCG.beta.'. The ligation product was transformed into DH5-alpha strain E. coli. and positive
clones were selected. Plasmid DNA was prepared by boiling lysis and digested with EcoNI and XhoI.
DNA which had the insert in the correct orientation produced fragments approximately 2.6 Kbp, 1.7
Kbp, 0.5 Kbp, 0.25 Kbp, and 0.15 Kbp. The plasmid DNA was then cotransfected into COS-7 cells
(obtained from the American Type Culture Collection) along with pSVL-hCG-alpha, a pSVL-based
plasmid encoding the alpha-subunit (10, 21), using a DEAE-dextran procedure (10, 21, 23, 24).
Beginning in 1-2 days and for a few days thereafter, the COS-7 cells produced significant amounts
of the free subunits and the heterodimer. These were present in the culture media and heterodimer
was detected using sandwich immunoassays employing monoclonal antibodies A113 and B105 (10). The
concentration of the protein was concentrated by ultrafiltration and monitored for its abilities
to bind to LH and FSH receptors by radioligand receptor assays using .sup.125 I-hCG and .sup.125
I-hFSH as tracers and rat ovarian corpora lutea and bovine testes as sources of LH and FSH
receptors as described (10).

25. Document ID: US 5466781 A

L8: Entry 25 of 43
File: USPT
Nov 14, 1995

US-PAT-NO: 5466781
DOCUMENT-IDENTIFIER: US 5466781 A
TITLE: Process for purifying bacterially produced M-CSF
DATE-ISSUED: November 14, 1995

US-CL-CURRENT: 530/351; 424/85.1, 435/69.5, 435/71.1, 435/71.2, 530/412, 530/414, 530/427

APPL-NO: 8/ 028375
DATE FILED: March 8, 1993

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation-in-part of U.S. patent
application Ser. No. 07/705,399, filed 24 May 1991 now abandoned.

IN: Dorin; Glenn, Gray; David R., Chang; Byeong S., Cowgill; Cynthia A., Milley;
Robert J.

AB: A process is described for producing M-CSF from bacteria. It includes:
fermentation of bacteria containing M-CSF DNA; harvest of the fractions that contain the
M-CSF protein (refractile bodies); primary recovery of the protein; solubilization and
denaturation of refractile bodies; M-CSF refolding; purification by column chromatography
and other methods; and formulation of the properly refolded M-CSF. This method is
advantageous over prior methods in terms of yield and purity.

L8: Entry 25 of 43
File: USPT
Nov 14, 1995

DOCUMENT-IDENTIFIER: US 5466781 A
TITLE: Process for purifying bacterially produced M-CSF

DEPR:
The resulting M-CSF monomer mixture is sterile filtered before anion exchange chromatography. For
this step, DEAE Zeta-Prep cartridges are preferred, but other resins that can be used are: PEI
(polyethylene imine) sold by FMC Corp; DEAE resin, sold by Pharmacia, for example; and CDR (cell
debris remover) sold by Whatman Biochemicals. When these resins are used at an increased ionic
strength, the nucleic acids stick to the resin, and the M-CSF monomer passes through the column.
This step removes residual nucleic acids and is more advantageous than the DEAE step shown in
'700, when run at a higher throughput, because more M-CSF monomer was lost in the old process.
This new step increases the ratio of M-CSF to nucleic acids, for example the plasmid DNA
concentration is reduced two fold. Specifically, DEAE Zeta-Prep separation in 50 mM NaCl, 8M
Urea, 12.5 mM EDTA, 5 mM DTT, 50 mM Tris at pH 8.5 is preferred. Instead of the anion exchange
column step, preferably, the higher molecular weight DNA is removed by ultrafiltration with a 300
kD MWCO membrane after the secondary oxidation. This diafiltration is also preferred because step
also removes high molecular weight M-CSF aggregates, which can lower M-CSF dimer yield in the
subsequent column steps. This filtration step eliminates the need for the DEAE column, making the
process more efficient on a large scale.

26. Document ID: US 5420029 A

L8: Entry 26 of 43
File: USPT
May 30, 1995

US-PAT-NO: 5420029
DOCUMENT-IDENTIFIER: US 5420029 A
TITLE: Mutated thermostable nucleic acid polymerase enzyme from thermotoga maritima
DATE-ISSUED: May 30, 1995

US-CL-CURRENT: 435/194; 536/23.2, 536/23.4

APPL-NO: 7/ 971819
DATE FILED: February 3, 1993

PARENT-CASE:
This application is a continuation-in-part of U.S. Ser. No. 07/567,244, filed Aug. 13, 1990, now
U.S. Pat. No. 5,374,553.

PCT-DATA:
APPL-NO
DATE-FILED
PUB-NO
PUB-DATE
371-DATE
102(E)-DATE
PCT/US91/05753

August 13, 1991
WO92/03556
Mar 5, 1992
Feb 3, 1993
Feb 3, 1993

IN: Gelfand; David H., Lawyer; Frances C., Stoffel; Susanne

AB: A purified thermostable enzyme is derived from the eubacterium Thermotoga maritima. The enzyme has a molecular weight as determined by gel electrophoresis of about 97 kilodaltons and DNA polymerase I activity. The enzyme can be produced from native or recombinant host cells and can be used with primers and nucleoside triphosphates in a temperature-cycling chain reaction where at least one nucleic acid sequence is amplified in quantity from an existing sequence.

L8: Entry 26 of 43
File: USPT
May 30, 1995

DOCUMENT-IDENTIFIER: US 5420029 A
TITLE: Mutated thermostable nucleic acid polymerase enzyme from thermotoga maritima

BSPR:
In the fifth stage, the fraction collected in the fourth stage is diluted with affigel-blue buffer without KCl and applied to an affigel-blue column equilibrated in 25 mM Tris-Cl (pH 7.5), 0.1 mM EDTA, 0.2% Tween 20, 0.5 mM DTT, and 0.15M KCl. The column is washed with the same buffer and eluted with a linear gradient of 0.15M to 0.7M KCl in the same buffer. The peak activity fractions were found at the 0.3M to 0.55M KCl section of the gradient. These fractions of peak activity are then tested for contaminating deoxyribonucleases (endonucleases and exonucleases) using any suitable procedure. As an example, endonuclease activity may be determined electrophoretically from the change in molecular weight of phage .lambda.. DNA or supercoiled plasmid DNA after incubation with an excess of DNA polymerase. Similarly, exonuclease activity may be determined electrophoretically from the change in molecular weight of restriction enzyme digested DNA after incubation with an excess of DNA polymerase. The fractions that have no deoxyribonuclease activity are pooled and diafiltered into phosphocellulose buffer containing 50 mM KCl.

27. Document ID: US 5316947 A

L8: Entry 27 of 43
File: USPT
May 31, 1994

US-PAT-NO: 5316947
DOCUMENT-IDENTIFIER: US 5316947 A
TITLE: Synthetic isohirudins with improved stability
DATE-ISSUED: May 31, 1994

US-CL-CURRENT: 435/320.1; 435/252.3, 435/252.33, 435/254.21, 435/69.1, 536/23.5

APPL-NO: 8/ 099053
DATE FILED: July 29, 1993

PARENT-CASE:
This is a division of application Ser. No. 07/985,110, filed Dec. 3, 1992.

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
DE 4140381 December 7, 1991

IN: Crause; Peter, Habermann; Paul, Tripier; Dominique, Ulmer; Wolfgang, Schmid; Gerhard

AB: Novel synthetic isohirudins with improved stability The invention relates to novel synthetic isohirudins which have improved stability owing to exchange in the region of the Asp-Gly motif. This results, on the one hand, in an increase in the yield during workup and, on the other hand, in making possible pharmaceutical formulation as directly injectable solution ready for use.

L8: Entry 27 of 43
File: USPT
May 31, 1994

DOCUMENT-IDENTIFIER: US 5316947 A
TITLE: Synthetic isohirudins with improved stability

DEPR:
A synthetic hirudin which has, in a modification of the natural sequence, a N-terminal amino acid leucine is described in European Patent Application EP-A 324 712. This hirudin can likewise be further optimized when the modifications described previously for the variants 13 and 93 are carried out in the sequence following leucine, from amino acid 2. In this connection, recourse is had by way of example to the vectors and strains described in this Application. The person skilled in the art is aware that every other yeast expression system which results in secretion of hirudin or variants thereof can also be used. The cloning vector 7 described in European Patent Application EP-A 324 712 is opened with BamHI and HindIII and in each case ligated to the BamHI/HindIII fragment which has been isolated from the plasmid pSCH13 or pSCH93 and which comprises amino acids of the carboxyl-terminal part of the hirudin sequence which are missing from the cloning vector in each case. The plasmids p713 and p793 are produced and are characterized by restriction analysis. Subsequently, the EcoRI/HindIII fragment is isolated from correct DNA of these plasmids, and the protruding ends are filled in a Klenow polymerase reaction. The fragments prepared in this way are ligated in each case to the blunt-ended vector fragment from the plasmid yEP13 as described in Example 1 of European Patent Application EP-A 324 712. The plasmids pHABVar131 and pHABVar132 which differ only with regard to the orientation of the inserted fragment and which code for a hirudin derivative which has the amino acids

Leu.sup.1, Glu.sup.33, Gln.sup.52, Glu.sup.53 and Glu.sup.55, and the plasmids pHABVar931 and
pHABVar932 which likewise differ only in the orientation of the inserted fragment and which code
for a hirudin derivative which has the amino acids Leu.sup.1, Glu.sup.33, Gln.sup.52, Glu.sup.53
and Ala.sup.54 are produced. The plasmids are, by way of example, transformed into the yeast
strains described in the Application. Expression and purification of the hirudin derivatives can
be carried out by the procedure described therein. It is known that it is possible in the
purification to dispense with centrifugation and subsequent adsorption chromatography when, for
example, the Millipore Pellicon ultrafiltration system is used. The methods used here are
described for the laboratory scale. For cultures on the cubic meter scale, other fermentation
times, culture conditions and steps in the workup may be necessary. This is known to the person
skilled in the art.

28. Document ID: US 5286714 A

L8: Entry 28 of 43 File: USPT Feb 15, 1994

US-PAT-NO: 5286714
DOCUMENT-IDENTIFIER: US 5286714 A
TITLE: Synthetic isohirudins with improved stability
DATE-ISSUED: February 15, 1994

US-CL-CURRENT: 514/12; 435/320.1, 435/69.1, 530/326, 530/416, 530/418, 536/23.5

APPL-NO: 7/ 985110
DATE FILED: December 3, 1992

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	APPL-DATE
DE	41 40 381.9	December 7, 1991

IN: Crause; Peter, Habermann; Paul, Tripier; Dominique, Ulmer; Wolfgang, Schmid; Gerhard

AB: The invention relates to novel synthetic isohirudins which have improved
stability owing to exchange in the region of the Asp-Gly motif. This results, on the one
hand, in an increase in the yield during workup and, on the other hand, in making possible
pharmaceutical formulation as directly injectable solution ready for use.

L8: Entry 28 of 43 File: USPT Feb 15, 1994

DOCUMENT-IDENTIFIER: US 5286714 A
TITLE: Synthetic isohirudins with improved stability

DEPR:
The cloning vector 7 described in European Patent Application EP-A 324 712 is opened with BamHI
and HindIII and in each case ligated to the BamHI/HindIII fragment which has been isolated from
the plasmid pSCH13 or pSCH93 and which comprises amino acids of the carboxyl-terminal part of the
hirudin sequence which are missing from the cloning vector in each case. The plasmids p713 and
p793 are produced and are characterized by restriction analysis. Subsequently, the EcoRI/HindIII
fragment is isolated from correct DNA of these plasmids, and the protruding ends are filled in a
Klenow polymerase reaction. The fragments prepared in this way are ligated in each case to the
blunt-ended vector fragment from the plasmid yEP13 as described in Example 1 of European Patent
Application EP-A 324 712. The plasmids pHABVar131 and pHABVar132 which differ only with regard to
the orientation of the inserted fragment and which code for a hirudin derivative which has the
amino acids Leu.sup.1, Glu.sup.33, Gln.sup.52, Glu.sup.53 and Glu.sup.55, and the plasmids
pHABVar931 and pHABVar932 which likewise differ only in the orientation of the inserted fragment
and which code for a hirudin derivative which has the amino acids Leu.sup.1, Glu.sup.33,
Gln.sup.52, Glu.sup.53 and Ala.sup.54 are produced. The plasmids are, by way of example,
transformed into the yeast strains described in the Application. Expression and purification of
the hirudin derivatives can be carried out by the procedure described therein. It is known that
it is possible in the purification to dispense with centrifugation and subsequent adsorption
chromatography when, for example, the Millipore Pellicon ultrafiltration system is used. The
methods used here are described for the laboratory scale. For cultures on the cubic meter scale,
other fermentation times, culture conditions and steps in the workup may be necessary. This is
known to the person skilled in the art.

29. Document ID: US 5242808 A

L8: Entry 29 of 43 File: USPT Sep 7, 1993

US-PAT-NO: 5242808
DOCUMENT-IDENTIFIER: US 5242808 A
TITLE: Production of bioadhesive precursor protein analogs by genetically engineered organisms
DATE-ISSUED: September 7, 1993

US-CL-CURRENT: 435/69.1; 435/252.3, 435/252.33, 435/254.21, 435/254.3, 435/320.1, 435/471,
435/69.7, 530/353

DISCLAIMER DATE: 20080917
APPL-NO: 7/ 644745
DATE FILED: January 23, 1991

PARENT-CASE:
CROSS-REFERENCE TO RELATED APPLICATIONS This application is a continuation of application Ser.
No. 07/025,243, filed Mar. 12, 1987, abandoned, which is a continuation-in-part of application
Ser. No. 06/933,945, filed Nov. 24, 1986, abandoned, which is a continuation-in-part of
application Ser. No. 06/650,128, filed Sep. 13, 1984, abandoned.

IN: Maugh; Kathy J., Anderson; David M., Strausberg; Susan L., Strausberg; Robert,
Wei; Tena

AB: Recombinant production of bioadhesive precursor protein analogs is disclosed. The
bioadhesive precursor protein analogs can be hydroxylated and used as an adhesive in wet
environments.

L8: Entry 29 of 43
File: USPT
Sep 7, 1993

DOCUMENT-IDENTIFIER: US 5242808 A
TITLE: Production of bioadhesive precursor protein analogs by genetically engineered organisms

DEPL:
After purification by preparative gel electrophoresis and reverse-phase chromatography, the
oligonucleotides were dissolved at a concentration of 1 delta 280 unit/ml. Oligonucleotides
#1876, #1988, and #1892 were phosphorylated individually in reactions with T.sub.4 polynucleotide
kinase and 1 mM ATP with 20 ul of oligonucleotide solution added in a 50 ul kinase reaction.
Oligonucleotides #1545 and #1546 were similarly treated, except they were pooled first at a 1:1
ratio. After the enzyme reaction, the solutions were boiled for two minutes to inactivate the
enzyme. An equivalent amount of oligonucleotide #1875 was added to the #1876 kinase reaction,
boiled for 30 seconds, then allowed to slow cool for formation of 5' linker Likewise, the #1892
and #1877 kinase reactions were mixed together with an equivalent amount of non-kinased #1893,
boiled, slow cooled and then ligated in a 180 ul volume at 16.degree. C. for 11 hours with
T.sub.4 polynucleotide ligase to assemble the 3' linker. Plasmid pGX2287 DNA (5 ug) was digested
with 18 units of ClaI endonuclease then extracted with phenol-chloroform, ethanol precipitated
and dissolved in 0.01 M Tris-HCl, 0.001 M EDTA (pH 8.0) at 0.25 ug DNA/ul. Ten microliters of the
ClaI-cut pGX2287 DNA was ligated with 25 ul of the 5' linker in a total volume of 40 ul at
16.degree. C. for 11 hours. After ligation, the DNA was phenol-chloroform extracted, ethanol
precipitated, then dissolved in 1 ml water. The DNA solution was concentrated using a Centricon
30 (Amicon) ultrafiltration unit, then washed two times with 2 ml water and centrifuged at 5,000
RPM for ten minutes. The washed and concentrated DNA, largely free of non-ligated linkers, was
ethanol precipitated and dissolved in 10 microliters of water.

30. Document ID: US 5232847 A

L8: Entry 30 of 43
File: USPT
Aug 3, 1993

US-PAT-NO: 5232847
DOCUMENT-IDENTIFIER: US 5232847 A
TITLE: Human tissue plasminogen activator analogue having substitutions at amino acid positions
66, 67 and 68
DATE-ISSUED: August 3, 1993

US-CL-CURRENT: 435/226; 424/94.63, 435/212, 435/219

APPL-NO: 7/ 613908
DATE FILED: December 11, 1990

FOREIGN-APPL-PRIORITY-DATA:
COUNTRY APPL-NO APPL-DATE
GB 8815135 June 24, 1988

PCT-DATA:
APPL-NO DATE-FILED PUB-NO PUB-DATE 371-DATE 102(E)-DATE
PCT/GB89/00705 June 23, 1989 WO89/12681 Dec 28, 1989 Dec 11, 1990 Dec 11, 1990

IN: Edwards; Richard M., Dawson; Keith, Fallon; Anthony, Craig; Stewart

AB: Tissue plasminogen activator (t-PA) analogues have at least one substitution in
the growth factor (GF) domain that at least partially reduces hepatic receptor binding
without substantially jeopardising physico-chemical stability in blood or fibrinolytic
activity. This results in a longer plasma half life. Substitutions in the beta-sheet
encompassing residues 63-72, especially at Leu 66 and/or Tyr 67 and/or Phe 68, are
particularly preferred.

L8: Entry 30 of 43
File: USPT
Aug 3, 1993

DOCUMENT-IDENTIFIER: US 5232847 A
TITLE: Human tissue plasminogen activator analogue having substitutions at amino acid positions
66, 67 and 68

DEPR:
TND-HBB plasmid DNA carrying the mutant t-PA gene was then linearised with the restriction
endonuclease XbaI and introduced into the non-secreting, non-producing mouse myeloma cell line
P3X63-Ag8.653 by electroporation. Plates yielding G418 resistant colonies were screened for t-PA
activity by using the indirect amidolytic assay involving activation of plasminogen in the
presence of fibrinogen and consequent cleavage of the chromogenic substrate S2251. Colonies
producing t-PA were then re-cloned and the best producers scaled up in flasks and then spinner
vessels to produce larger amounts of the t-PA derivative. t-PA was purified from the conditioned
medium by affinity chromatography using Erythrina trypsin inhibitor immobilised on CNBr activated
SEPHAROSE CL4B followed by elution using 3 M KSCN, desalting on

SEPHADEX G25 and concentration by
ultrafiltration. (The words SEPHAROSE and SEPHADEX are trade marks.) The purified t-PA derivative
was then assayed for specific activity using the S2251 assay and assessed for receptor binding by
its ability to compete with I.sup.125 labelled t-PA for binding to rat liver hepatocytes. The in
vivo efficacy at clot lysis was determined in a rabbit femoral artery model that also permitted
the measurement of the plasma half-life as determined by following both amidolytic activity and
t-PA antigen using an ELISA assay.

31. Document ID: US 5149657 A

L8: Entry 31 of 43
File: USPT
Sep 22, 1992

US-PAT-NO: 5149657
DOCUMENT-IDENTIFIER: US 5149657 A
TITLE: Escherichia coli expression vector encoding bioadhesive precursor protein analogs
comprising three to twenty repeats of the decapeptide (Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys)
DATE-ISSUED: September 22, 1992

US-CL-CURRENT: 435/320.1; 435/252.33, 435/69.1, 435/69.7, 530/353

DISCLAIMER DATE: 20080917
APPL-NO: 7/ 655234
DATE FILED: February 8, 1991

PARENT-CASE:
CROSS REFERENCE TO RELATED APPLICATIONS This application is a continuation of application Ser.
No. 07/025,140, filed Mar. 12, 1987 now abandoned, which is a continuation in part of U.S. Ser.
No. 06/671,967, filed Nov. 16, 1984 now U.S. Pat. No. 4,798,791; and U.S. Ser. No. 06/933,945,
filed Nov. 24, 1986, now abandoned, which is a continuation-in-part of U.S. Ser. No. 06/650,128,
filed Sep. 13, 1984, now abandoned.

IN: Maugh; Kathy J., Anderson; David M.

AB: Recombinant production of a bioadhesive precursor protein analog comprising
three, five, ten, fifteen or twenty repeated decapeptides of the formula Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys is disclosed.

L8: Entry 31 of 43
File: USPT
Sep 22, 1992

DOCUMENT-IDENTIFIER: US 5149657 A
TITLE: Escherichia coli expression vector encoding bioadhesive precursor protein analogs
comprising three to twenty repeats of the decapeptide (Ala-Lys-Pro-Ser-Tyr-Pro-Pro-Thr-Tyr-Lys)

DEPR:
Plasmid pGX2287 DNA (5 ug) was digested with 18 units of ClaI endonuclease then extracted with
phenol-chloroform, ethanol precipitated and dissolved in 0.01M Tris-HCl, 0.001M EDTA (pH 8.0) at
0.25 ug DNA/ul. Ten microliters of the ClaI-cut pGX2287 DNA was ligated with 25 ul of the 5'
linker in a total volume of 40 ul at 16.degree. C. for 11 hours. After ligation, the DNA was
phenol-chloroform extracted, ethanol precipitated, then dissolved in 1 ml water. The DNA solution
was concentrated using a Centricon 30 (Amicon) ultrafiltration unit, then washed two times with 2
ml water and centrifuged at 5,000 RPM for ten minutes. The washed and concentrated DNA, largely
free of non-ligated linkers, was ethanol precipitated and dissolved in 10 microliters of water.

32. Document ID: US 5047505 A

L8: Entry 32 of 43
File: USPT
Sep 10, 1991

US-PAT-NO: 5047505
DOCUMENT-IDENTIFIER: US 5047505 A
TITLE: High level expression in E. coli of soluble mature HIL-1beta and derivatives with altered
biological activity
DATE-ISSUED: September 10, 1991

US-CL-CURRENT: 530/351; 435/69.52, 536/23.51

APPL-NO: 7/ 132185
DATE FILED: December 18, 1987

PARENT-CASE:
CROSS-REFERENCE This application is a continuation-in-part of co-pending application Ser. No.
006,870, filed Jan. 27, 1987, now abandoned.

IN: Huang; James J.

AB: Plasmid pUC8 and DNA coding for hIL-1.beta. are used to construct hybrid plasmids
capable of high level expression in E. coli of soluble proteins, including mature
hIL-1.beta. and derivatives of mature hIL-1.beta. having amino acid substitutions and
insertions at one or all of positions 1 to 4 at the amino terminus. Derivatives of
hIL-1.beta. with alterations at the N-terminus have been produced which have either enhanced
or decreased bioactivity compared to native monocyte derived hIL-1.beta..

L8: Entry 32 of 43
File: USPT
Sep 10, 1991

DOCUMENT-IDENTIFIER: US 5047505 A
TITLE: High level expression in E. coli of soluble mature HIL-1beta and derivatives with altered
biological activity

DEPR:
E. coli cells of the ampicillin-sensitive JM 101 strain were transformed with plasmid DNA. Cells
were grown in L Broth supplemented with ampicillin, (100 .mu.g/ml) and IPTG
(isopropeylthio-.beta.-galactoside at 37.degree. C. in a rotary shaker (150 rpm). Recombinant
clones were grown to a Klett reading of 30 (determined by

Klett-Summerson Photoelectric
Colorimeter, Klett Manufacturing Company, New York) at which time IPTG was added to a final
concentration of 1 mM. Cells were harvested at various time points for further characterization.
Cells from 500 ml culture were harvested, resuspended in 50 ml of sonication buffer (50 mM Tris
pH 8.0, 1 mM EDTA, 1 mM DTT) and sonicated for 7 to 10 seconds in a 5 ml volume. Sonicated
samples were centrifuged for 5 minutes at 4.degree. C. The supernatants and pellets were kept
separate. Sonicated lysate was filtered with a Millipore filter (0.45.mu.) before it was applied
to SYNCHROPAK Ion Exchange Column (2.1.times.25 cm) from Synchrom, Inc., Linden, Ind. The
hIL-1.beta. containing fractions in this and subsequent chromatography steps were identified by
Western Blot assay using a rabbit polyclonal antibody against monocyte hIL-1.beta.. Fractions
containing hIL-1.beta. were pooled and concentrated to 1 to 3 ml by ultrafiltration with an
AMICON concentrator and were further purified by ACA sizing column chromatography (2.4.times.100
cm) from LKB Instruments, Inc., Gaithersburg, MD. Buffer used in both columns was 50 mM Tris pH
8, 1 mM EDTA, 1 mM DTT. Native form hIL-1.beta. was purified from the myleomonocytic THP-1 cell
line as described by Matsushima et al. Biochem. 25:3424-3429 (1986).

09/121,798
Att#18

Set Items Description
--- ----- -----------
? s plasmid(2n)DNA

196099 PLASMID
1653821 DNA
S1 36941 PLASMID(2N)DNA
? s static(w)mixer

57461 STATIC
2050 MIXER
S2 78 STATIC(W)MIXER
? s ultrafilt? or diafilt?

34461 ULTRAFILT?
971 DIAFILT?
S3 35057 ULTRAFILT? OR DIAFILT?
? s s1 and s2

36941 S1
78 S2
S4 4 S1 AND S2
? s s1 and s3

36941 S1
35057 S3
S5 48 S1 AND S3
? rd s4

...completed examining records
S6 4 RD S4 (unique items)
? rd s5

...completed examining records
S7 42 RD S5 (unique items)
? s s6 or s7

4 S6
42 S7
S8 46 S6 OR S7
? t s8/3,ab/1-46

8/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

12551829 BIOSIS NO.: 200000305331
Methods for purifying nucleic acids.
AUTHOR: Bussey Lee B(a); Adamson Robert; Atchley Alan
AUTHOR ADDRESS: (a)San Mateo, CA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office
Patents 1230 (1):pNo pagination Jan. 4, 2000
MEDIUM: e-file
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Methods are provided for producing highly purified compositions of nucleic acids by using tangential flow %%%ultrafiltration%%%. A scaleable process for producing pharmaceutical grade %%%plasmid%%% %%%DNA%%%, useful for gene therapy, is provided, which is efficient and avoids the use of toxic organic chemicals.

2000

8/3,AB/2 (Item 2 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

12338123 BIOSIS NO.: 200000091625
Production of %%%plasmid%%% %%%DNA%%% for human gene therapy using modified alkaline cell lysis and expanded bed anion exchange chromatography.
AUTHOR: Varley D L; Hitchcock A G; Weiss A M E; Horler W A; Cowell R; Peddie L; Sharpe G S; Thatcher D R; Hanak J A J(a)
AUTHOR ADDRESS: (a)Cobra Therapeutics, Science Park, Keele, Staffs., ST5 5SP**UK
JOURNAL: Bioseparation 8 (1-5):p209-217 1999
ISSN: 0923-179X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English
SUMMARY LANGUAGE: English

ABSTRACT: We describe a process for the commercial manufacture of therapeutic grade %%%plasmid%%% %%%DNA%%%. The industrially scaleable unit operations employed in this process are: (i) optimized alkaline lysis; (ii) bag filtration; (iii) expanded bed anion exchange chromatography; (iv) %%%ultrafiltration%%%, and (v) size exclusion chromatography. These steps are scaleable alternatives to current approaches to %%%plasmid%%% %%%DNA%%% isolation such as high speed centrifugation for feed-stock clarification and solvent precipitation for plasmid concentration, and an efficient alternative to conventional low through-put packed bed chromatography. The process produces %%%plasmid%%% %%%DNA%%% characterized by low level chromosomal DNA, RNA and endotoxin contamination without the use of flammable solvents or toxic reagents and is suitable for therapeutic administration.

1999

8/3,AB/3 (Item 3 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

11894706 BIOSIS NO.: 199900140815
Bacteriocin production by Pseudomonas syringae pv. ciccaronei NCPPB2355.
Isolation and partial characterization of the antimicrobial compound.
AUTHOR: Lavermicocca P(a); Lonigro S L; Evidente A; Andolfi A
AUTHOR ADDRESS: (a)Istituto Tossine e Micotossine da Parassiti vegetali, C.N.R., V. le L. Einaudi 51, I-70125, Bari**Italy
JOURNAL: Journal of Applied Microbiology 86 (2):p257-265 Feb., 1999
ISSN: 1364-5072
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Pseudomonas syringae pv. ciccaronei strain NCPPB2355 was found to produce a bacteriocin inhibitory against strains of Ps. syringae subsp. savastanoi, the causal agent of olive knot disease. Treatments with mitomycin C did not substantially increase the bacteriocin titre in culture. The purification of the bacteriocin obtained by ammonium sulphate precipitation of culture supernatant fluid, membrane %%%ultrafiltration%%%, gel filtration and preparative PAGE, led to the isolation of a high molecular weight proteinaceous substance. The bacteriocin analysed by SDS-PAGE revealed three protein bands with molecular weights of 76, 63 and 45 kDa, respectively. The bacteriocin was sensitive to heat and proteolytic enzymes, was resistant to non-polar organic solvents and was active between pH 5.0-7.0. %%%Plasmid%%% %%%DNA%%% analysis of Ps. syringae ciccaronei revealed the presence of 18 plasmids; bacteriocin-negative variants could not be obtained by cure experiments.

1999

8/3,AB/4 (Item 4 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

11640471 BIOSIS NO.: 199800422202

%%%Ultrafiltration%%% on %%%plasmid%%% %%%DNA%%%: Characterization and
optimization.
AUTHOR: Clark David W; Semsler Jim
AUTHOR ADDRESS: Process Dev. Manufacturing, Apollon Inc., One Great Valley
Parkway, Malvern, PA**USA
JOURNAL: Abstracts of Papers American Chemical Society 216 (1-3):pBIOT 156
1998
CONFERENCE/MEETING: 216th National Meeting of the American Chemical Society
Boston, Massachusetts, USA August 23-27, 1998
SPONSOR: American Chemical Society
ISSN: 0065-7727
RECORD TYPE: Citation
LANGUAGE: English
1998

8/3,AB/5 (Item 5 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

10433621 BIOSIS NO.: 199699054766
Analysis of ligase chain reaction products via matrix-assisted laser desorption/ionization time-of-flight-mass spectrometry.
AUTHOR: Jurinke Christian; Van Den Boom Dirk; Jacob Anette; Tang Kai; Woerl
Ralf; Koester Hubert(a)
AUTHOR ADDRESS: (a)Dep. Biochemistry Molecular Biol., Fac. Chem., Univ.
Hamburg, Martin-Luther-King-Platz 6, D-2014**Germany
JOURNAL: Analytical Biochemistry 237 (2):p174-181 1996
ISSN: 0003-2697
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A rapid and accurate detection of ligation products generated in ligase chain reactions (LCR) by using matrix-assisted laser desorption/ionization time-offlight-mass spectrometry (MALDI-TOF-MS) is reported. LCR with Pfu DNA ligase was performed with a wild-type template
and a template carrying a single point mutation within the Escherichia coli lacI gene as a model system. Starting from about 1 fmol of template DNA the ligation product generated in the positive reactions was analyzed with HPLC and MALDI-TOFMS, whereby the need of proper sample purification
prior to mass spectrometric analysis was demonstrated. A purification procedure with a high potential for automation using streptavidin-coated magnetic particles and %%%ultrafiltration%%% was introduced. %%%Plasmid%%% %%%DNA%%% and short single-stranded oligonucleotides have
been used as template. A point mutation could be discriminated from the wild-type template due to the absence or presence of ligation product. This approach allows the rapid-specific detection of template DNA in femtomole amounts and moreover can distinguish between sequence variations in DNA molecules down to point mutations without the need for labeling, gel electrophoresis, membrane transfer, or hybridization procedures.

1996

8/3,AB/6 (Item 6 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)
(c) 2001 BIOSIS. All rts. reserv.

09721909 BIOSIS NO.: 199598176827
Continuously coupled transcription-translation system for the production of rice cytoplasmic aldolase.
AUTHOR: Tulin Edgardo E; Tsutsumi Ken-Ichi; Ejiri Shin-Ichiro(a)
AUTHOR ADDRESS: (a)Inst. Cell Biol. Genetics, Fac. Agric., Iwate Univ. Ueda, Morioka, Iwate 020**Japan
JOURNAL: Biotechnology and Bioengineering 45 (6):p511-516 1995
ISSN: 0006-3592
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A continuously coupled cell-free transcription-translation system
was developed for the production of rice cytoplasmic aldolase, an enzyme involved in both glycolytic and gluconeogenic pathways in eukaryotic cells. The system works with a continuous flow of feeding solution containing nucleoside triphosphates and amino acids into a 1-mL reactor containing wheat-germ extract, %%%plasmid%%% %%%DNA%%%, and transcription
enzyme, and continuous removal of translation product through an %%%ultrafiltration%%% membrane fitted in the reactor. Addition of free nucleotide primer, m-7G(5')ppp(5')G, to this reactor was necessary for efficient transcription, thus producing biologically active mRNA for translation. The rate of aldolase synthesis was constant during the continuous translation reaction. It was observed that from 3 h onward only aldolase was synthesized by the system. After 30 h, the total amount of protein synthesized reached 205.6 mu-g, which is comparable with the amount synthesized (255.6 mu-g) in the translation system only where separately prepared capped mRNAs were added to the reactor for translation. Autoradiogram and Western blot analyses of the translated product showed a distinct band corresponding to the calculated molecular weight of the protein. These results have shown the establishment of a continuously coupled eukaryotic transcription-translation system for the expression of genes from eukaryotic cells.

1995

8/3,AB/7 (Item 1 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2001 Elsevier Science B.V. All rts. reserv.

07785592 EMBASE No: 1999260751
Design of multi-functional nanoparticles as a DNA carrier
Maruyama A.; Ishihara T.; Kim J.-S.; Wan Kim S.; Akaike T.
A. Maruyama, Dept. Biomolecular Engineering, Faculty of Bioscience/Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midori, Yokohama 226-5081 Japan
Colloids and Surfaces A: Physicochemical and Engineering Aspects (COLLOIDS SURF. A PHYSICOCHEM. ENG. ASP.) (Netherlands) 1999, 153/1-3
(439-443)
CODEN: CPEAE ISSN: 0927-7757
PUBLISHER ITEM IDENTIFIER: S0927775798005342
DOCUMENT TYPE: Journal; Conference Paper
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH
NUMBER OF REFERENCES: 12

Novel biodegradable nanoparticles which contain the sites for both polynucleotide adsorption and targeting ligand on their surfaces were prepared as a carrier for genetic materials. The nanoparticles were obtained from poly(D,L-lactic acid) and poly(L-lysine)-graft-polysaccharide copolymers by using a %%%diafiltration%%% method. The size of the particles
was controlled by varying the initial concentration of the graft copolymer or by changing the polysaccharide content in the graft copolymers. Polysaccharide moieties on the surface of the nanoparticles were found to interact specifically with a corresponding lectin. The number of amino groups on the nanoparticles surfaces increased with increasing polysaccharides content in the graft copolymers, suggesting that grafted polysaccharide chains modify the adsorption conformation of poly(L-lysine) moiety in the graft copolymer on the nanoparticle surface. Both %%%plasmid%%% %%%DNA%%% and oligonucleotide (40 mer) were adsorbed stably
on the nanoparticles surfaces through the ionic interaction between polynucleotides and poly(L-lysine) segments without inducing inter-particle aggregation. Our results suggest that the nanoparticles prepared from poly(D,L-lactic acid) and poly(L-lysine)-graft-polysaccharide copolymer basically form a multi-layered structure composed of polysaccharide-rich surface, poly(L-lysine)-rich intermediate, and poly(D,L-lactic acid)-cored inner layers. The nanoparticles offer several advantages such as ease in DNA loading, stable dispersiveness in aqueous media, and polysaccharide-based surface functionality, implying usefulness of the particles as a carrier and/or controlled release matrix of polynucleotides.

8/3,AB/8 (Item 2 from file: 73)
DIALOG(R)File 73:EMBASE
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05405282 EMBASE No: 1993173381
Gene expression in cell-free system on preparative scale
Baranov V.I.; Spirin A.S.
Methods in Enzymology (METHODS ENZYMOL.) (United States) 1993, 217/-
(123-142)
CODEN: MENZA ISSN: 0076-6879
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH

8/3,AB/9 (Item 3 from file: 73)
DIALOG(R)File 73:EMBASE
(c) 2001 Elsevier Science B.V. All rts. reserv.

05377399 EMBASE No: 1993145498
A simple improvement to the Triton lysis procedure for plasmid isolation
Huang A.; Campbell J.
Naval Research Laboratory,Washington, DC 20375 United States
BioTechniques (BIOTECHNIQUES) (United States) 1993, 14/5 (730)
CODEN: BTNQD ISSN: 0736-6205
DOCUMENT TYPE: Journal; Article
LANGUAGE: ENGLISH

8/3,AB/10 (Item 1 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09821380 99150978
Large scale purification of %%%plasmid%%% %%%DNA%%% for use in gene
therapy.
Ollivier M; Stadler J
Rhone-Poulenc Rorer, GENCELL, Vitry sur Seine, France.
Advances in experimental medicine and biology (UNITED STATES) 1998,
451 p487-92, ISSN 0065-2598 Journal Code: 2LU
Languages: ENGLISH
Document type: JOURNAL ARTICLE

8/3,AB/11 (Item 2 from file: 155)
DIALOG(R)File 155:MEDLINE(R)
(c) format only 2000 Dialog Corporation. All rts. reserv.

09273491 97248708
Simple and rapid preparation of plasmid template by a filtration method using microtiter filter plates.
Itoh M; Carninci P; Nagaoka S; Sasaki N; Okazaki Y; Ohsumi T; Muramatsu M
; Hayashizaki Y
Genome Science Laboratory, Tsukuba Life Science Center, The Institute of Physical and Chemical Research (RIKEN), Koyadai 3-1-1, Tsukuba-city, Ibaraki 305, Japan.
Nucleic acids research (ENGLAND) Mar 15 1997, 25 (6) p1315-6, ISSN
0305-1048 Journal Code: O8L
Languages: ENGLISH
Document type: JOURNAL ARTICLE
We developed a new simple high-throughput %%%plasmid%%% %%%DNA%%%
extraction procedure, based on a modified alkaline lysis method, using only one 96-well microtiter glassfilter plate. In this method, cell harvesting, lysis by alkaline and plasmid purification are performed on only one microtiter glassfilter plate. After washing out RNAs or other contaminants, %%%plasmid%%% %%%DNA%%% is eluted by low-ion strength solution, although
precipitated chromosomal DNA is not eluted. The plasmid prepared by this method can be applied to sequencing reactions or restriction enzyme cleavage.

8/3,AB/12 (Item 1 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
(c) 2001 Derwent Publ Ltd. All rts. reserv.

0254558 DBA Accession No.: 2000-09048
Purification of %%%plasmid%%% %%%DNA%%% by tangential flow filtration -
Factor-VIII %%%plasmid%%% %%%DNA%%% purification from Escherichia coli
by alkaline lysis and lysozyme
AUTHOR: Kahn D W; Butler M D; Cohen D L; Gordon M; Kahn J W; Winkler M
E
CORPORATE AFFILIATE: Genentech Cohen-Sci.Consult.
CORPORATE SOURCE: Department of Recovery Science, Genentech Inc., 1 DNA
Way, South San Francisco, CA, USA. email:kahn.david@gene.com
JOURNAL: Biotechnol.Bioeng. (69, 1, 101-06) 2000
ISSN: 0006-3592 CODEN: BIBIAU
LANGUAGE: English
ABSTRACT: A method for purification of %%%plasmid%%% %%%DNA%%% by
tangential flow filtration was developed. A plasmid carrying the gene for Factor-VIII was transformed into Escherichia coli and cultured in a 10 l fermentor and treated with cycloheximide to maximize production of plasmid. Cells were lyzed by alkaline lysis using a lysozyme (EC-3.2.1.17) solution of 0.8 ml (2 mg/ml in GTE) and incubated for 30 min on ice. A supernatant was obtained and used for purification of %%%plasmid%%% %%%DNA%%% by tangential flow filtration. The tangential
flow membrane was 1,000,000 Da with an area of 0.5 ft sq. of a polyethersulfone membrane per 10 to 15 g of cells processed. Experiments indicated that the %%%ultrafiltration%%% membranes required
15 to 20 min of initial operation with the clarified supernatant under normal operating conditions prior to initiation of %%%ultrafiltration%%% to minimize initial yield losses in filtrate. themethod typically yielded 15 to 20 mg of %%%plasmid%%% %%%DNA%%% per
l of bacterial culture and resulted in removal of more than 99% of RNA and more than 95% of the protein that remained after the alkaline lysis procedure. (24 ref)

8/3,AB/13 (Item 2 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0251017 DBA Accession No.: 2000-05507 PATENT
Purifying nucleic acids from bacterial cells using static mixers for lysing cells and precipitating debris, followed by centrifugation and ionexchange chromatography - vector plasmid p4119 purification from Escherichia coli using cell disintegration for use in cloning, polymerase chain reaction-mediated diagnosis and gene therapy
AUTHOR: Bridenbaugh R; Dang W; Bussey L
CORPORATE SOURCE: Burlingame, CA, USA.
PATENT ASSIGNEE: Valentis 2000
PATENT NUMBER: WO 200005358 PATENT DATE: 20000203 WPI ACCESSION NO.:
2000-171430 (2015)
PRIORITY APPLIC. NO.: US 121798 APPLIC. DATE: 19980723
NATIONAL APPLIC. NO.: WO 99US15280 APPLIC. DATE: 19990707
LANGUAGE: English
ABSTRACT: A new method for purifying %%%plasmid%%% %%%DNA%%% from bacterial
cells which consists of cell disintegration in a %%%static%%% %%%mixer%%% , precipitation and centrifugtion to isolate the clarified solution containing the %%%plasmid%%% %%%DNA%%% , which is then
neutralized and contacted with a positively charged ionexchange chromatography resin in order to obtain a solution containing the purified %%%plasmid%%% %%%DNA%%%, is claimed. The %%%plasmid%%%
%%%DNA%%% (nucleic acid) purified using this method may be useful for a
variety of applications such as molecular biological applications, e.g. cloning or gene expression, or for diagnostic applications, using polymerase chain reaction (PCR), reverse transcription-PCR, dendromer formation, etc., or for therapeutic uses, e.g. in gene therapy. In an

example, vector plasmid p4119 was isolated from Escherichia coli cells with a final yield of 80%. This new method minimizes complex and expensive purification steps, but it yields high quality DNA and so is economical. It may be used to produce pharmaceutical grade %%%plasmid%%% %%%DNA%%%. (35pp)

8/3,AB/14 (Item 3 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0243945 DBA Accession No.: 1999-14710
Bacteriocin production by Pseudomonas syringae pv. ciccaronei NCPPB2355.
Isolation and partial purification of the antimicrobial compound - for application as olive knot disease biological control agent
AUTHOR: Lavermicocca P; Lonigro S L; Evidente A; Andolfi A
CORPORATE AFFILIATE: Univ.Naples
CORPORATE SOURCE: Istituto Tossine e Micotossine da Parassiti Vegetali,
CNR, V. le L. Einaudi 51, I-70125 Bari, Italy.
email:p.lavermicocca@area.ba.cnr.it
JOURNAL: J.Appl.Microbiol. (86, 2, 257-65) 1999
ISSN: 1364-5072 CODEN: JAMIFK
LANGUAGE: English
ABSTRACT: Pseudomonas syringae pv. ciccaronei NCPPB2355 produced a bacteriocin inhibitory against strains of P. syringae subsp. savastanoi, the causal agent of olive knot disease. Cells were cultured at 26 deg with 100 rpm in 250 ml Erlenmeyer flasks with 100 ml of specified media. Treatments with mitomycin-C did not substantially increase the bacteriocin titer in the culture. The purification of the bacteriocin obtained by ammonium sulfate precipitation of culture supernatant fluid, membrane %%%ultrafiltration%%%, gel filtration and preparative PAGE, led to the isolation of a high mol.wt. proteinaceous substance. The bacteriocin analyzed by SDS-PAGE revealed 3 protein bands with mol.wt. values of 76,000, 63,000 and 45,000, respectively. The bacteriocin was sensitive to heat and proteolytic enzymes, was resistant to non-polar organic solvents and was active between pH 5 and 7. %%%Plasmid%%% %%%DNA%%% analysis of P. syringae ciccaronei revealed the presence of 18 plasmids; bacteriocin-negative variants could not be obtained by cure experiments. The bacteriocin may be used as a biological control agent. (28 ref)

8/3,AB/15 (Item 4 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0239288 DBA Accession No.: 99-09389 PATENT
Preparation of polynucleotide transfection complexes, using a dual-feed process - nucleic acid transfection complex preparation by mixing polycation, e.g. liposome, with nucleic acid, for potential gene therapy
AUTHOR: Bridenbaugh R; Dang W; Koe G
CORPORATE SOURCE: Burlingame, CA, USA.
PATENT ASSIGNEE: Megabios 1999
PATENT NUMBER: WO 9922009 PATENT DATE: 990506 WPI ACCESSION NO.:
99-303021 (9925)
PRIORITY APPLIC. NO.: US 94437 APPLIC. DATE: 980728
NATIONAL APPLIC. NO.: WO 98US22518 APPLIC. DATE: 981023
LANGUAGE: English
ABSTRACT: A means of producing a nucleic acid transfection complex is claimed. It involves providing a solution of a nucleic acid and a polycation in a 1st and 2nd feed stream respectively. The feed streams are then mixed to allow formation of the nucleic acid transfection complex. This is used for the delivery of nucleic acids to cells, particularly eukaryotic cells, in vitro and in vivo. The process is highly reproducible, and scaleable. The nucleic acid is preferably DNA, and the polycation is preferably a cationic lipid, polylysine, polyarginine or polyhistidine, particularly one that contains a polycationic lipid and a neutral lipid. The streams are mixed in a %%%static%%% %%%mixer%%%. In an example, %%%plasmid%%% %%%DNA%%% was diluted to 0.5 mg/ml and liposomes were diluted to 20 mM. Equal volumes of DNA and liposomes were combined into a single feed stream at inlet flow rates of 80 ml/minute with a linear flow rate of 0.45 feet/second. (0pp)

8/3,AB/16 (Item 5 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0231234 DBA Accession No.: 99-01335 PATENT
Method for lysing cells while avoiding the shearing of genomic DNA - cell disintegration using %%%static%%% %%%mixer%%%, by simultaneously flowing cell suspension fluid and lysis solution through mixer, used for %%%DNA%%% purification and %%%plasmid%%% rescue
AUTHOR: Wan N C; McNeilly D S; Christopher C W
CORPORATE SOURCE: Cambridge, MA, USA.
PATENT ASSIGNEE: Genzyme 1998
PATENT NUMBER: US 5837529 PATENT DATE: 981117 WPI ACCESSION NO.:
99-023457 (9902)
PRIORITY APPLIC. NO.: US 632203 APPLIC. DATE: 960415
NATIONAL APPLIC. NO.: US 632203 APPLIC. DATE: 960415
LANGUAGE: English
ABSTRACT: A method and apparatus for cell disintegration with the avoidance of shearing genomic DNA is claimed and comprises a mixer through which a fluid containing the cell suspension and a cell lysing solution are simultaneously flowed. Also claimed is the separation of plasmids from plasmid containing cells using the same method. The method is used for DNA purification, and rapidly lyses large amounts of cells (multi-gram amounts) to produce undamaged DNA. It is effective, economical and automatable, and makes large scale biological procedures involving cell lysis more feasible. The %%%static%%% %%%mixer%%% may be used as part of a series of mixers, and may also include a precipitation step whereby the lysate is combined with a precipitating solution prior to entry into a third %%%static%%% %%%mixer%%% in a series. The purified %%%DNA%%% or %%%plasmid%%% may be used in gene therapy. (8pp)

8/3,AB/17 (Item 6 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0225641 DBA Accession No.: 98-07238 PATENT
Production of nucleic acid conjugates - %%%plasmid%%% %%%DNA%%% and RNA
conjugate preparation for use in gene transfer and sense, antisense gene expression control
AUTHOR: Bayer E; Fritz H; Maier M
CORPORATE SOURCE: Trostberg, Germany.
PATENT ASSIGNEE: SKW-Trostberg 1998
PATENT NUMBER: DE 19746362 PATENT DATE: 980430 WPI ACCESSION NO.:
98-252414 (9823)
PRIORITY APPLIC. NO.: DE 1046362 APPLIC. DATE: 971021
NATIONAL APPLIC. NO.: DE 1046362 APPLIC. DATE: 971021
LANGUAGE: German
ABSTRACT: A new process for the production of conjugates of nucleic acids with polymer nanoparticles involves subjecting sparingly water-soluble vinylic monomers to emulsion polymerization in an aq. medium in the presence of a cationic radical initiator and in the absence of an emulsifier, preferably purifying the suspension by %%%diafiltration%%% or centrifugation, and reacting the resulting polymer suspension with a nucleic acid at 10-30 deg and pH less than 11. The conjugates are useful for gene transfer or for sense or antisense control of gene expression. Conjugates with high nucleic acid loadings and adequate resistance to enzyme degradation can be produced. The monomers preferably have a water solubility below 20 g/l and are selected from styrene, acrylic acid derivatives and methacrylic acid derivatives. The polymer suspension has a particle size of 10-1,000 nm. The nucleic acid is optionally chemically modified DNA or RNA with a length of 7-40 nucleotides, and is preferably a plasmid. (5pp)

8/3,AB/18 (Item 7 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0223558 DBA Accession No.: 98-05155 PATENT
Purification of nucleic acid by %%%ultrafiltration%%% and collection of retentate - DNA purification
AUTHOR: Bussey L B; Adamson R; Atchley A
CORPORATE SOURCE: Burlingame, CA, USA.
PATENT ASSIGNEE: Megabios 1998
PATENT NUMBER: WO 9805673 PATENT DATE: 980212 WPI ACCESSION NO.:
98-145547 (9813)
PRIORITY APPLIC. NO.: US 691090 APPLIC. DATE: 960801
NATIONAL APPLIC. NO.: WO 97US13493 APPLIC. DATE: 970731
LANGUAGE: English
ABSTRACT: Nucleic acid (I) is purified from solution by filtering it through an %%%ultrafiltration%%% unit comprising a gel layer and collecting the (I)-containig retentate solution. Also new are: recovery of %%%plasmid%%% %%%DNA%%% from solution by filtration through an open channel %%%ultrafiltration%%% unit having a membrane of mol.wt. cut-off 50,000-500,000 and collecting the %%%plasmid%%% %%%DNA%%%-containing retentate; and purification of %%%plasmid%%% %%%DNA%%% from a mixture of cells by lysing cells in surfactant-containing buffer, digesting the cellular RNA enzymatically in the solubilized cell solution, differentially precipitating (and removing) cellular debris and proteins, and purifying the %%%plasmid%%% %%%DNA%%% -containing supernatant by tangential flow %%%ultrafiltration%%% and collecting the retentate containing the %%%plasmid%%% %%%DNA%%%. The method is useful for purifying virus or %%%plasmid%%% %%%DNA%%% or RNA, especially phjarmaceutical grade plasmids for use in gene therapy (to express therapeutic proteins, antisense molecules or ribozymes). (39pp)

8/3,AB/19 (Item 8 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0220778 DBA Accession No.: 98-02375 PATENT
New %%%DNA%%% fragments, a %%%plasmid%%% carrying them, a recombinant microbe and preparation of a trimmed enzyme by using it - e.g. Bacillus sp. recombinant thermostable acid cellulase truncated mutant expression in Bacillus subtilis
CORPORATE SOURCE: Japan.
PATENT ASSIGNEE: Amano-Pharm. 1997
PATENT NUMBER: JP 9271385 PATENT DATE: 971021 WPI ACCESSION NO.:
98-003022 (9801)
PRIORITY APPLIC. NO.: JP 96134492 APPLIC. DATE: 960401
NATIONAL APPLIC. NO.: JP 96134492 APPLIC. DATE: 960401
LANGUAGE: JA
ABSTRACT: A new DNA fragment containing a truncated enzyme gene gives a restriction map. Also claimed are a vector containing the DNA fragment, a recombinant microbe transformed with the above DNA, and a method for the preparation of a truncated enzyme in which the microbe is cultured and the truncated enzyme is collected. The method may be used to prepare a truncated enzyme of wide substrate specificity having heat resistance and efficient activity in acid conditions. In an example, Bacillus sp. APC-9603 was cultured in MB medium at 37 deg for 65 hr. The culture was centrifuged and the supernatant was concentrated by %%%ultrafiltration%%%. The concentrate was subjected to alpha-CD Sepharose column chromatography. The eluate was dialyzed and purified and the corresponding gene was cloned. A recombinant plasmid insert was subcloned and a cellulase (EC-3.2.1.4) gene was cloned and expressed in Bacillus subtilis. (15pp)

8/3,AB/20 (Item 9 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0216600 DBA Accession No.: 97-11721
Characterization of %%%plasmid%%% %%%DNA%%% vectors for use in human gene therapy, part 1 - %%%plasmid%%% characterization by %%%DNA%%% sequencing, chromatography, microscopy, etc.
AUTHOR: Marquet M; Horn N A; Meek J A
CORPORATE AFFILIATE: Vical
CORPORATE SOURCE: Vical Inc., 9373 Towne Centre Drive, San Diego, CA 92121, USA. email:biotech@ix.netcom.com
JOURNAL: Biopharm Manuf. (10, 5, 42-44,46,48,50) 1997
ISSN: 1040-8304 CODEN: BPRME5
LANGUAGE: English
ABSTRACT: Several methods for characterizing %%%plasmid%%% %%%DNA%%% products in terms of identity, purity and potency are discussed and compared with methods with analogous measurements used to characterize recombinant proteins. A diagram is presented which shows the partition of impurities across a %%%plasmid%%% %%%DNA%%% manufacturing process involving cell lysis, %%%diafiltration%%% concentration, plasmid precipitation and chromatography as the main production process with branches showing the waste e.g. host cell DNA and protein, RNA and salts obtained at different points in the process. Characterization of %%%plasmid%%% %%%DNA%%% may be performed by physicochemical methods (complete sequence analysis, restriction endonuclease analysis, linear flow dichroism, electrophoresis, chromatography (HPLC), spectroscopy and microscopy (atomic force microscopy and scanning force microscopy). Contaminants tested for include pyrogens, proteins and antigens, cellular DNA contaminants, RNA contaminants, microbial contamination and residuals. (37 ref)

8/3,AB/21 (Item 10 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0216131 DBA Accession No.: 97-11252 PATENT
Nucleic acid preparations of low protein and endotoxin contents - %%%plasmid%%% vector %%%DNA%%% purification for use in ex vivo or in vivo gene therapy
AUTHOR: Kuhne W
CORPORATE SOURCE: Mannheim, Germany.
PATENT ASSIGNEE: Boehr.Mannheim 1997
PATENT NUMBER: WO 9729113 PATENT DATE: 970814 WPI ACCESSION NO.:
97-415287 (9738)
PRIORITY APPLIC. NO.: EP 96101628 APPLIC. DATE: 960206
NATIONAL APPLIC. NO.: WO 97EP321 APPLIC. DATE: 970124
LANGUAGE: German
ABSTRACT: A new nucleic acid preparation contains less than 0.1% protein and less than 1 (preferably 0.01-0.1) EU endotoxin per mg DNA. The preparation is also free of ethidium bromide, phenol cesium chloride, MOPS buffer and polyethoxylated octylphenol-based surfactant. The DNA is preferably a plasmid which replicates in Gram-negative bacteria. The DNA is replicated in a bacterium host, followed by lysis of biomass and hydroxyapatite chromatography of soluble components. Endotoxin and nucleic acid components bind to the adsorbent by dipole-dipole interactions, typically from neutral phosphate buffer. The column is washed with a solution containing denaturant, e.g. urea, and the nucleic acid is eluted, particularly with denaturant-free 0.2-0.5 M phosphate, while the endotoxin remains bound. The eluate is preferably subjected to %%%ultrafiltration%%% or ethanol-isopropanol precipitation. The preparation may be used for in vivo or ex vivo gene therapy. (14pp)

8/3,AB/22 (Item 11 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs

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0214756 DBA Accession No.: 97-09877 PATENT
Lysing cells using static mixers - cell disintegration by simultaneous flow with lytic solution, precipitation solution and/or %%%plasmid%%% %%%DNA%%% purification solution, for use in gene therapy vector or nucleic acid vaccine production
AUTHOR: Wan N C; McNeilly D S; Christopher C W
CORPORATE SOURCE: Cambridge, MA, USA.
PATENT ASSIGNEE: Genzyme 1997
PATENT NUMBER: WO 9723601 PATENT DATE: 970703 WPI ACCESSION NO.:
97-351044 (9732)
PRIORITY APPLIC. NO.: WO 95US16843 APPLIC. DATE: 951221
NATIONAL APPLIC. NO.: WO 95US16843 APPLIC. DATE: 951221
LANGUAGE: English
ABSTRACT: A new cell lysis method involves simultaneous flow of a cell suspension and a lytic solution through a %%%static%%% %%%mixer%%%,
where cells are lysed on exit. The lytic solution may contain an alkali, surfactant, organic solvent and/or a lytic enzyme. The cells preferably contain a plasmid. A precipitating solution (containing e.g. SDS and/or potassium acetate) may be added to the lysate during flow to precipitate cellular components. The method may be used to release plasmids in soluble form. The method may be used in preparation of therapeutic DNA for e.g. gene therapy of genetic disease, or use as a nucleic acid vaccine. The method may be used in treatment of multi-liter amounts of solution containing multi-gram amounts of cells. The cells may be lysed rapidly, making large-scale biological procedures involving cell disintegration feasible. In an example, Escherichia coli cells grown at high cell density were passed through a Kenics %%%static%%% %%%mixer%%% along with 50 mM Tris-HCl, 10 mM EDTA
and 100 mg/ml RNA-ase-A, then with 200 mM NaOH and 1% SDS, and then
with 2.6 M potassium acetate, pH 5.2, to give 1 g %%%plasmid%%%
%%%DNA%%%. (17pp)

8/3,AB/23 (Item 12 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0213904 DBA Accession No.: 97-09025
Direct transfection of polymerase chain reaction-generated DNA fragments into mammalian cells employing ethidium bromide indicator and %%%ultrafiltration%%% - e.g. estrogen receptor gene 5'-region or %%%plasmid%%% pGEX-2TK %%%DNA%%% fragment gene transfer to MCF-7 cell
culture, for use in e.g. gene therapy
AUTHOR: Penolazzi L; Facciolo M C; Aguiari G; del Senno L; Piva R
CORPORATE AFFILIATE: Univ.Ferrara
CORPORATE SOURCE: Dipartimento di Biochimica e Biologia Molecolare,
Universita Degli Studi di Ferrara, Via L. Borsari, 46, 44100 Ferrara, Italy.
JOURNAL: Anal.Biochem. (248, 1, 190-93) 1997
ISSN: 0003-2697 CODEN: ANBCA2
LANGUAGE: English
ABSTRACT: A method was developed for direct transfection of human MCF-7
cells with DNA fragments obtained by polymerase chain reaction (PCR), using %%%ultrafiltration%%% and an ethidium bromide label, and could be
adapted for delivery of small ds DNA fragments, and to evaluate efficiency of DNA uptake by cells. A 96-bp human genomic fragment from
the 5'-region of the estrogen receptor gene and a 150-bp %%%plasmid%%%
pGEX-2TK %%%DNA%%% fragment were used to test the system. Fragments
were obtained by 30 cycles of PCR with 2.5 U Taq DNA-polymerase (EC-2.7.7.7), followed by labeling of PCR products with ethidium bromide. Free label was removed by %%%ultrafiltration%%%, and fluorescence-labeled DNA was incubated with 100,000 cells for 2 or 24 hr at 37 deg. After transfection for 24 hr, fluorescence was clearly detectable in 90% of cells, showing a good transfection efficiency, but cells incubated for 2 hr showed no fluorescence, indicating that DNA uptake was slow. These PCR fragments may be useful alternative to oligonucleotides for use in gene therapy. (11 ref)

8/3,AB/24 (Item 13 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0207713 DBA Accession No.: 97-02834
Properties and gene structure of the Thermotoga maritima alpha-amylase AmyA, a putative lipoprotein of a hyperthermophilic bacterium - recombinant thermostable enzyme preparation by vector expression in Escherichia coli, purification and characterization
AUTHOR: Liebl W; Stemplinger I; Ruile P
CORPORATE AFFILIATE: Univ.Munich-Tech.
CORPORATE SOURCE: Lehrstuhl fuer Mikrobiologie, Technische Universitaet
Muenchen, Arcisstr. 21, D-80290 Munich, Germany.
JOURNAL: J.Bacteriol. (179, 3, 941-48) 1997
ISSN: 0021-9193 CODEN: JOBAAY
LANGUAGE: English
ABSTRACT: Recombinant Escherichia coli JM83 clones from a Thermotoga
maritima MSB8 DNA library constructed in plasmid pUN121 were screened
for thermostable alpha-amylase (EC-3.2.1.1) activity. Five positive clones were identified, the plasmids purified from which contained largely overlapping %%%DNA%%% insert fragments.
%%%Plasmid%%% pUCTAA29
carried all sequence information necessary for expression of the thermostable alpha-amylase and was therefore chosen for further examination. The alpha-amylase protein was purified from E. coli JM83(pUCTAA29) grown aerobically in LB broth supplemented with 12 ug/ml
oxytetracycline. Culture broth (about 15 l) was centrifuged and cells were lyzed using a French pressure cell. Heat-labile host proteins were precipitated by treatment of the crude lyzate with heat (75 deg) and removed by centrifugation. The supernatant was dialyzed and applied to a Q-Sepharose Fast Flow HR 10/10 column. Active fractions were dialyzed
and subjected to Mono-Q HR 5/5 column chromatography, followed by
%%%ultrafiltration%%%. The recombinant enzyme had a mol.wt. of about
61,000 by denaturing gel electrophoresis, a pH optimum of 7.0 and an optimum temp. of 85-90 deg. (38 ref)

8/3,AB/25 (Item 14 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0206344 DBA Accession No.: 97-01465 PATENT
Large scale purification of %%%plasmid%%% %%%DNA%%% - from Escherichia coli
fermentation using a heat exchanger, anion-exchange chromatography and reverse-phase HPLC for application in nucleic acid vaccine and gene therapy
AUTHOR: Lee A L; Sagar S
CORPORATE SOURCE: Rahway, NJ, USA.
PATENT ASSIGNEE: Merck-USA 1996
PATENT NUMBER: WO 9636706 PATENT DATE: 961121 WPI ACCESSION NO.:
97-020828 (9701)
PRIORITY APPLIC. NO.: US 446118 APPLIC. DATE: 950519
NATIONAL APPLIC. NO.: WO 96US7083 APPLIC. DATE: 960515
LANGUAGE: English
ABSTRACT: A process for large-scale %%%plasmid%%% %%%DNA%%% isolation and
purification from a microbial cell (e.g. Escherichia coli) fermentation is new and involves: harvesting the cells from a 33.7 l fermentation; adding a lysis solution (STET buffer containing 8% sucrose, 2% Triton, 50 mM Tris buffer, 50 mM EDTA and lysozyme (EC-3.2.1.17, pH 8.5) to the
cells; heating the cells to 70-100 deg in a flow-through heat exchanger to form a crude lysate; centrifuging the crude lysate for 50 mins at 9,000 rpm; filtering and %%%diafiltering%%% the supernatant; contacting

the filtrate with an anion-exchange matrix; eluting and collecting %%%plasmid%%% %%%DNA%%% from the matrix; subjecting the %%%plasmid%%% %%%DNA%%% to reverse-phase HPLC; optionally concentrating and/or %%%diafiltering%%% the product into a carrier; and optionally sterilizing the DNA product. Also claimed is an isolated and purified %%%plasmid%%% %%%DNA%%% (2,110 mg) suitable for administration to humans and non-human animals. The product can be used in nucleic acid vaccines for human gene therapy. (33pp)

8/3,AB/26 (Item 15 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0193789 DBA Accession No.: 96-05196 PATENT
Large-scale isolation and purification of %%%plasmid%%% %%%DNA%%% - DNA purification for use in genetic immunization or gene therapy
AUTHOR: Lee A L; Sagar S
CORPORATE SOURCE: Rahway, NJ, USA.
PATENT ASSIGNEE: Merck-USA 1996
PATENT NUMBER: WO 9602658 PATENT DATE: 960201 WPI ACCESSION NO.: 96-105920 (9611)
PRIORITY APPLIC. NO.: US 275571 APPLIC. DATE: 940715
NATIONAL APPLIC. NO.: WO 95US8749 APPLIC. DATE: 950711
LANGUAGE: English
ABSTRACT: A new process for large-scale purification of %%%plasmid%%% %%%DNA%%% from large-scale microbial fermentations involves harvesting cells, resuspension in lysis buffer, heating to 70-100 deg in a flow-through heat exchanger to form a crude lysate, centrifugation, filtration, %%%diafiltration%%% , anion-exchange chromatography, reverse-phase HPLC and optional concentration and/or %%%diafiltration%%% into a pharmaceutically acceptable adsorbent, or sterilization. The lysis buffer is preferably modified STET buffer, with a sub-ug concentration of lysozyme (EC-3.2.1.17). Lysis is preferably carried out at 70-77 deg. The method may include RNA-ase treatment at any stage after harvesting of cells. The plasmid is preferably for administration to humans or animals, for genetic immunization or gene therapy. The method allows large-scale commercially viable preparation of %%%plasmid%%% %%%DNA%%%, and does not require hazardous or expensive chemicals, e.g. ethidium bromide. The method is less labor-intensive and time-consuming than previous methods, and gives greater yields, with inactivation of endogenous DNA-ases. (33pp)

8/3,AB/27 (Item 16 from file: 357)
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0192583 DBA Accession No.: 96-02776
cGMP production and quality assurance of %%%plasmid%%% %%%DNA%%% used for human gene therapy and nucleic acid vaccination - vector production for e.g. genetic immunization; quality control; new %%%ultrafiltration%%% apparatus (conference abstract)
AUTHOR: Schorr J; Moritz P; Schleef M
CORPORATE AFFILIATE: Qiagen
CORPORATE SOURCE: QIAGEN GmbH, Max-Volmer-Strasse 4, D-40724 Hilden, Germany.
JOURNAL: Gene Ther. (2, Suppl.1, S12) 1995
ISSN: 0969-7128 CODEN: 4352W
CONFERENCE PROCEEDINGS: Human Gene Transfer and Therapy, 3rd Meeting, Barcelona, Spain, 17-20 November, 1995.
LANGUAGE: English
ABSTRACT: A prerequisite for plasmid-mediated gene therapy or genetic immunization is that the %%%plasmid%%% %%%DNA%%% itself should not induce an immune response. Injected DNA must be free of all contamination, particularly toxic or antigenic substances. Pyrogenic lipopolysaccharides (endotoxins) are common components of Gram-negative bacterial cell walls, which must be completely removed from %%%plasmid%%% %%%DNA%%% used in therapy, transfection or microinjection. For large-scale DNA production, the Ultrapure 100 anion-exchange column has been designed for preparation of up to 100 mg of ultrapure %%%plasmid%%% %%%DNA%%% . In combination with special buffers and a new filtration device, the Ultrapure 100 column produces endotoxin-free DNA without time-consuming centrifugation steps. DNA purified by the new method is assayed for endotoxin, RNA, protein, genome DNA, homogeneity (at least 90% CCC) and sterility. To optimize %%%plasmid%%% %%%DNA%%% quality, Escherichia coli strains and culture media have been tested. The new method has been approved to produce DNA for human clinical phase-I studies in the UK and other European countries, and in the USA by the FDA. (4 ref)

8/3,AB/28 (Item 17 from file: 357)
DIALOG(R)File 357:Derwent Biotechnology Abs
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0184455 DBA Accession No.: 95-11276 PATENT
Recombinant plasmid vector for pig somatotropin production - gene cloning and expression in e.g. Bacillus subtilis
PATENT ASSIGNEE: Daicel-Chem. 1995
PATENT NUMBER: JP 7155183 PATENT DATE: 950620 WPI ACCESSION NO.: 95-250733 (9533)
PRIORITY APPLIC. NO.: JP 93339300 APPLIC. DATE: 931202
NATIONAL APPLIC. NO.: JP 93339300 APPLIC. DATE: 931202
LANGUAGE: JA
ABSTRACT: A new recombinant plasmid vector contains a hybrid pig somatotropin gene, consisting of a Bacillus sp. alpha-amylase (EC-3.2.1.1) protein secretion signal peptide and a pig somatotropin structural gene, located downstream from the signal sequence, and with a promoter for Bacillus sp. upstream from the hybrid gene. The pig somatotropin gene (an EcoRI-BamHI fragment) is linked to a site between EcoRI and BamHI sites of an Escherichia coli plasmid, and used to transform an appropriate host, preferably Bacillus subtilis. The recombinant somatotropin is purified by conventional methods, e.g. centrifugation, salting-out, solvent precipitation, %%%ultrafiltration%%%% %%% %%% , SDS-agarose gel electrophoresis, ionexchange chromatography, etc.%%% %%% The vector may be used to produce pig somatotropin in Bacillus spp. The%%% %%% gene product may be mass-produced by this method, and is in a native%%% %%% form which is effective and safe in pigs. (13pp)%%% %%%%%% %%%%%% %%% 8/3,AB/29 (Item 18 from file: 357)%%% %%%DIALOG(R)File 357:Derwent Biotechnology Abs%%% %%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%% %%%%%% %%%0159155 DBA Accession No.: 94-01706%%% %%%Chloroperoxidase-encoding gene from Pseudomonas pyrrocinia: sequence%%% %%% expression in heterologous hosts, and purification of the enzyme -%%% %%% expression in Streptomyces lividans, and recombinant enzyme%%% %%% purification from Escherichia coli; DNA sequence%%% %%%AUTHOR: Wolfframm C; Lingens F; Mutzel R; +van Pee K H%%% %%%CORPORATE AFFILIATE: Univ.Hohenheim-Inst.Microbiol. Univ.Konstanz%%% %%%CORPORATE SOURCE: Institut fuer Mikrobiologie, Universitaet Hohenheim,%%% %%% Garbenstr. 30, D-7000 Stuttgart 70, Germany.%%% %%%JOURNAL: Gene (130, 1, 131-35) 1993%%% %%%CODEN: GENED6%%% %%%LANGUAGE: English%%% %%%ABSTRACT: The DNA sequence of a 1.5-kb fragment of

Pseudomonas pyrrocinia%%%
%%% %%%DNA%%% from %%%plasmid%%% pHW321, containing a chloroperoxidase%%%
%%% (CPO, EC-1.11.1.10) cpo gene and its flanking regions, was determined.%%%
%%% The enzyme may be useful in production of chlorinated organic%%%
%%% compounds. The cpo gene encoded a protein of 278 amino acids. The%%%
%%% mature enzyme contained no N-terminal Met residue, so that the CPO%%%
%%% monomer consisted of 277 amino acids (mol.wt. 30,304). The cpo gene was%%%
%%% functionally expressed in Escherichia coli (using pHW321) and%%%
%%% Streptomyces lividans TK64 (using plasmid pHW322). The enzyme was%%%
%%% overproduced in E. coli to levels of 3,535 U/g wet wt. cells on%%%
%%% induction with IPTG. The recombinant enzyme was purified 4.8-fold by a%%%
%%% new method, which involved anion-exchange chromatography on%%%
%%% DEAE-Sephacel, hydrophobic interaction chromatography on%%%
%%% phenyl-Sepharose and %%%ultrafiltration%%%, to give a yield of 49% and%%%
%%% a specific activity of 63 U/mg, and gave 800-fold more CPO/g cells than%%%
%%% from P. pyrrocinia. The enzyme had 38% protein sequence identity with%%%
%%% Streptomyces aureofaciens ATCC 10762 bromoperoxidase-A2. (23 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/30 (Item 19 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
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%%%%%%%
%%%0154197 DBA Accession No.: 93-12249%%%
%%%Cloning and sequence analysis of the meso-diaminopimelate-decarboxylase%%%
%%% gene from Bacillus methanolicus MGA3 and comparison to other%%%
%%% decarboxylase genes - gene cloning and expression in Escherichia coli%%%
%%% for use in L-lysine preparation%%%
%%%AUTHOR: Mills D A; Flickinger M C%%%
%%%CORPORATE SOURCE: Department of Biochemistry, 240 Gortner Laboratories,%%%
%%% 1479 Gortner Avenue, University of Minnesota, St. Paul, Minnesota%%%
%%% 55108, USA.%%%
%%%JOURNAL: Appl.Environ.Microbiol. (59, 9, 2927-37) 1993%%%
%%%CODEN: AEMIDF%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: The diaminopimelate-decarboxylase (DAPD, EC-4.1.1.20) lysA gene%%%
%%% of the industrial L-lysine-producing strain Bacillus methanolicus MGA3%%%
%%% was cloned by complementation of Escherichia coli AT2452, a lysA22%%%
%%% auxotrophic mutant, with an MGA3 chromosomal DNA gene bank in a plasmid%%%
%%% pBR322 vector. The lysA gene was subcloned as a 2.3-kb SmaI-SstI%%%
%%% fragment using plasmid pUC19cm as a vector, to form %%%plasmid%%% pDM5.%%%
%%% The %%%DNA%%% sequence of the 2.3-kb insert was determined, and%%%
%%% contained an open reading frame encoding a protein of mol.wt. 48,223,%%%
%%% with a sequence similar to other DAPDs. The recombinant enzyme was%%%
%%% purified by ammonium sulfate precipitation, anion-exchange%%%
%%% chromatography on DEAE-agarose, %%%ultrafiltration%%% and%%%
%%% hydroxyapatite chromatography. The B. methanolicus DAPD was a dimer%%%
%%% (mol.wt. 86,000) with a subunit mol.wt. of 50,000. The enzyme has%%%
%%% inhibited by lysine (Ki 0.93 mM), with a Km of 0.8 mM for%%%
%%% diaminopimelic acid. The inhibition pattern suggested that the activity%%%
%%% of this enzyme in lysine-overproducing strains of B. methanolicus may%%%
%%% limit lysine biosynthesis. (47 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/31 (Item 20 from file: 357)%%%
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%%%%%%%
%%%0153677 DBA Accession No.: 93-11729 PATENT%%%
%%%Isolated and purified bacteriocin from Lactococcus lactis subsp. lactis -%%%
%%% gene cloning in Lactococcus spp. for use in food%%%
%%%PATENT ASSIGNEE: Quest-Int. 1993%%%
%%%PATENT NUMBER: US 5231165 PATENT DATE: 930727 WPI ACCESSION NO.:%%%
%%% 93-249768 (9331)%%%
%%%PRIORITY APPLIC. NO.: US 882715 APPLIC. DATE: 920514%%%
%%%NATIONAL APPLIC. NO.: US 882715 APPLIC. DATE: 920514%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: A new polypeptide inhibits sensitive Gram-positive bacteria, and%%%
%%% has a defined protein sequence and encoding DNA sequence. The%%%
%%% polypeptide is bacteriocin-LL-2 from Lactococcus lactis subsp. lactis%%%
%%% NRRL B-18809, and may be purified by %%%ultrafiltration%%% ,%%%
%%% anion-exchange chromatography and lyophilization. A gene encoding the%%%
%%% polypeptide may be cloned in other Lactococcus spp. using L. lactis%%%
%%% subsp. lactis plasmid pSRQ400 (69 kb). The amount of bacteriocin%%%
%%% required to provide inhibition is 15-100 arbitrary units/g material.%%%
%%% The bacteriocin is useful in food or non-food materials, and inhibits%%%
%%% Lactobacillus plantarum, Lactobacillus casei, Lactobacillus brevis,%%%
%%% Lactobacillus bulgaricus, Lactobacillus fermentum, Pediococcus%%%
%%% acidilactici, Pediococcus pentosaceus, Streptococcus mutans, Bacillus%%%
%%% subtilis and L. lactis. (13pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/32 (Item 21 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0151021 DBA Accession No.: 93-09073%%%
%%%Gene expression in cell-free systems on a preparative scale - recombinant%%%
%%% protein production by in vitro translation or transcription-translation%%%
%%%AUTHOR: Spirin A S%%%
%%%CORPORATE SOURCE: Institute of Protein Research, Russian Academy of%%%
%%% Sciences, Pushchino, Moscow Region, Russia.%%%
%%%JOURNAL: Bioorg.Khim. (18, 10-11, 1395-402) 1992%%%
%%%CODEN: BIKHD7%%%
%%%LANGUAGE: Russian%%%
%%%ABSTRACT: Large-scale recombinant protein production by in vitro%%%
%%% translation or transcription-translation systems is discussed.%%%
%%% Cell-free systems overcome in vivo problems of

low-efficiency%%%
%%% expression, protein aggregation, protease degradation and product%%%
%%% toxicity. %%%Ultrafiltration%%% chambers containing mixtures of%%%
%%% ribosomes, templates, tRNA, protein factors, etc., may be used, and the%%%
%%% product diffuses out as nutrient solution is added. For 1 ml reactions,%%%
%%% flow rates of 1-3 ml/hr are used, and reactions are stable for 20-100%%%
%%% hr. Reaction components are provided by cell extracts (S30%%%
%%% supernatants) with endogenous or added mRNA or %%%plasmid%%% %%%DNA%%%,%%%
%%% or from purified components, and may be from prokaryote (e.g.%%%
%%% Escherichia coli) or eukaryote (e.g. wheat germ or rabbit reticulocyte)%%%
%%% cells. Examples of in vitro translation include phage MS2 RNA%%%
%%% translation, tobacco mosaic virus protein production and calcitonin%%%
%%% production. Examples of transcription-translation systems include%%%
%%% production of beta-lactamase (EC-3.5.2.6), dihydrofolate-reductase%%%
%%% (EC-1.5.1.3) and chloramphenicol-acetyltransferase (EC-2.3.1.28) from%%%
%%% cloned genes. (5 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/33 (Item 22 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0143852 DBA Accession No.: 93-01904 PATENT%%%
%%%%%%%Plasmid%%% %%%DNA%%% and/or cosmid DNA purification from microorganism%%%
%%% cells - by cell lysis, filtration to remove insoluble material and%%%
%%% %%%ultrafiltration%%%%%%
%%%PATENT ASSIGNEE: Tosoh 1992%%%
%%%PATENT NUMBER: EP 517515 PATENT DATE: 921209 WPI ACCESSION NO.: 92-408948%%%
%%%(9250)%%%
%%%PRIORITY APPLIC. NO.: JP 91159436 APPLIC. DATE: 910604%%%
%%%NATIONAL APPLIC. NO.: EP 92305119 APPLIC. DATE: 920604%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: A new method for %%%plasmid%%% %%%DNA%%% and/or cosmid DNA%%%
%%% purification from microorganism cells, which have been transformed or%%%
%%% transfected, comprises: cell lysis; filtration of the resulting lyzate%%%
%%% through a membrane filter to remove any insoluble material; and%%%
%%% subjecting the filtrate to %%%ultrafiltration%%% to condense the DNA.%%%
%%% The pore size of the membrane filter is 0.1-2 um, and/or the mol.wt. to%%%
%%% be fractionated by %%%ultrafiltration%%% is 30,000-1,000,000.%%%
%%% Preferably, microbial RNA is removed by treating the purified DNA on%%%
%%% the %%%ultrafilter%%% with a solution containing RNA-ase, or by washing%%%
%%% the %%%ultrafilter%%% with a solution containing either RNA-ase or aq.%%%
%%% alkaline metal hydroxide (NaOH and/or KOH and/or a surfactant such as%%%
%%% SDS). Preferably the lyzate is neutralized e.g. with acid prior to%%%
%%% filtration. Preferably, the microbial cells are those of Escherichia%%%
%%% coli, and/or the DNA to be purified is derived from plasmid pUC119 or%%%
%%% plasmid pBluescript. The purified DNA is also new. The purification is%%%
%%% simple, does not require toxic reagents, and gives highly purified DNA.%%%
%%% (5pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/34 (Item 23 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0137231 DBA Accession No.: 92-09723 PATENT%%%
%%%Recombinant plasmid for dihydrofolate-reductase-antiallergic pentapeptide%%%
%%% fusion protein production - gene cloning and expression in Escherichia%%%
%%% coli; fusion protein cleavage%%%
%%%PATENT ASSIGNEE: Agency-Ind.Sci.; Hitachi-Chem. 1992%%%
%%%PATENT NUMBER: JP P4117284 PATENT DATE: 920417 WPI ACCESSION NO.:%%%
%%% 92-180076 (9222)%%%
%%%PRIORITY APPLIC. NO.: JP 90123201 APPLIC. DATE: 900515%%%
%%%NATIONAL APPLIC. NO.: JP 90123201 APPLIC. DATE: 900515%%%
%%%LANGUAGE: Japanese%%%
%%%ABSTRACT: A recombinant %%%plasmid%%% containing a %%%DNA%%% sequence%%%
%%% encoding a dihydrofolate-reductase (I, EC-1.5.1.3)-antiallergic%%%
%%% pentapeptide (II) fusion protein (specified protein sequence) is%%%
%%% claimed. Escherichia coli transformed by the recombinant plasmid%%%
%%% contains a (I)-(II) fusion protein with a protein sequence. The%%%
%%% transformed bacteria can produce and accumulate the (I)-(II) fusion%%%
%%% protein in a large amount in soluble form, and it can be easily%%%
%%% purified. The peptide is prepared by cleaving the fusion protein and is%%%
%%% used as a drug to treat allergic diseases. In an example, plasmid%%%
%%% pBK10MM was prepared by combining it to E. coli HB101, and the fusion%%%
%%% protein was obtained from the transformed E. coli by extraction. The%%%
%%% fusion protein was analyzed by SDS-PAGE to show a single band of%%%
%%% 21,000. It was concentrated by %%%ultrafiltration%%% and dialysis, and%%%
%%% then cleaved with cyanogen bromide at room temp. overnight and fed%%%
%%% through a HPLC (YMC-ODS-5) column, the linear gradient was eluted with%%%
%%% 0 to 10 % acetonitrile and the peptide was recovered at 44% yield.%%%
%%% (12pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/35 (Item 24 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0132013 DBA Accession No.: 92-04505 PATENT%%%
%%%New cytotoxic protein obtained from Pichia inositovora - killer toxin with%%%
%%% fungicide, insecticide, nematocide and herbicide activity%%%
%%%PATENT ASSIGNEE: Agr.Res.Serv.Washington 1991%%%
%%%PATENT NUMBER: US 7733512 PATENT DATE: 911210 WPI ACCESSION NO.:%%%
%%% 92-049393 (9206)%%%
%%%PRIORITY APPLIC. NO.: US 733512 APPLIC. DATE: 910722%%%
%%%NATIONAL APPLIC. NO.: US 733512 APPLIC. DATE: 910722%%%
%%%LANGUAGE: English%%%

%%%ABSTRACT: A new killer toxin protein is produced by Pichia inositovora NRRL%%%
%%% Y-18709. The strain contains 3 linear %%%DNA%%% plasmids, designated%%%
%%% %%%plasmid%%% pPin1-1 (18 kb), plasmid pPin1-2 (13 kb) and plasmid%%%
%%% pPin1-3 (10 kb). The protein may also be produced by transformants,%%%
%%% especially yeasts, containing an expression vector with a gene encoding%%%
%%% the protein. The protein may be used as a fungicide in therapy of%%%
%%% fungal infections, and for prevention and control of fungal growth. The%%%
%%% protein may also be used as an insecticide, nematocide or herbicide. In%%%
%%% an example, P. inositovora was grown in 10 flasks, each with 1.5 l YEPD%%%
%%% culture medium (1.5 ml inoculum/1.5 l), overnight at 25-29 deg with%%%
%%% agitation. The protein was purified from culture supernatant by%%%
%%% %%%ultrafiltration%%% (mol.wt. 100,000 cutoff), dialysis (mol.wt.%%%
%%% 12,000-14,000 cutoff), filter sterilization and gel filtration HPLC on%%%
%%% Bio-Sil SEC-400. The mol.wt. of the purified protein was 160,000 +/-%%%
%%% 50,000. (15pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/36 (Item 25 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0130713 DBA Accession No.: 92-03205%%%
%%%Purification and analysis of proteinase-resistant mutants of recombinant%%%
%%% platelet-derived growth factor-BB exhibiting improved biological%%%
%%% activity - human recombinant protein production; artificial gene%%%
%%% cloning and vector plasmid pSW6 expression in Saccharomyces cerevisiae;%%%
%%% protein secretion%%%
%%%AUTHOR: Cook A L; Kirwin P M; Craig S; Bawden L J; Green D R; Price M%%%
%%% J%%%
%%%CORPORATE AFFILIATE: Brit.Bio-technol.%%%
%%%CORPORATE SOURCE: British Bio-technology Ltd., Watlington Road, Cowley,%%%
%%% Oxford OX4 5LY, UK.%%%
%%%JOURNAL: Biochem.J. (281, Pt.1, 57-65) 1992%%%
%%%CODEN: BIJOAK%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: A synthetic gene for human platelet-derived growth factor-B%%%
%%% (PDGF-BB) was constructed with codon usage optimized for Saccharomyces%%%
%%% cerevisiae, and a number of introduced restriction sites. The sequence%%%
%%% encoded a 160 amino acid protein from the N-terminus of mature PDGF-BB,%%%
%%% which ended with a natural translational stop codon. A PDGF-BB gene%%%
%%% with the mature C-terminus was constructed by deleting from Thr-109 to%%%
%%% the translational stop codon. The N-terminal sequence of the synthetic%%%
%%% genes was modified by addition of an oligonucleotide linker to enable%%%
%%% the fusion of the mature sequence to that of the yeast pre-pro-alpha%%%
%%% factor gene. The alpha factor sequence facilitated secretion of%%%
%%% recombinant PDGF-B from protease-deficient yeast strain BJ2168, after%%%
%%% its transformation with expression vector plasmid pSW6. The mature%%%
%%% PDGF-BB was liberated from the pre-pro sequence by the action of the%%%
%%% yeast KEX2 lysine-arginine endopeptidase. Substitution of Arg-28 or%%%
%%% Arg-32 prevented cleavage and gave 5-fold higher expression levels. The%%%
%%% protein was purified 95% by %%%ultrafiltration%%%, and CM-Sepharose and%%%
%%% phenyl-Sepharose chromatography. (57 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/37 (Item 26 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0124292 DBA Accession No.: 91-11934%%%
%%%A single-strand specific endonuclease activity copurifies with%%%
%%% overexpressed T5 D15-exonuclease - phage T1 gene cloning and expression%%%
%%% in Escherichia coli; potential application in site-directed mutagenesis%%%
%%% and %%%plasmid%%% %%%DNA%%% purification%%%
%%%AUTHOR: Sayers J R; +Eckstein F%%%
%%%CORPORATE AFFILIATE: Max-Planck-Inst.Exp.Med.%%%
%%%CORPORATE SOURCE: Max-Planck-Institut fuer Experimentelle Medizin,%%%
%%% Abteilung Chemie, Hermann-Rein-Strasse 3, W-3400 Goettingen, Germany.%%%
%%%JOURNAL: Nucleic Acids Res. (19, 15, 4127-32) 1991%%%
%%%CODEN: NARHAD%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: A gene encoding phage T5 D15-exonuclease was cloned and%%%
%%% over-expressed in Escherichia coli M72 (phage lambda), using plasmid%%%
%%% pJONEX44 as vector, and the recombinant enzyme was purified by%%%
%%% anion-exchange FPLC on Mono Q, hydroxyapatite chromatography, P 11%%%
%%% phosphocellulose chromatography, cation-exchange chromatography on%%%
%%% SP-Sephadex C-25 and CM-Sephadex, hydrophobic interaction%%%
%%% chromatography on phenyl Sepharose CL-4B, controlled pore glass%%%
%%% chromatography; gel filtration chromatography on Sephadex G75,%%%
%%% %%%ultrafiltration%%%, isoelectric focusing, and preparative SDS-PAGE.%%%
%%% The enzyme showed a low level of endonuclease activity, specific for%%%
%%% single-stranded DNA, when assayed with 1-10 mM Mg2+ as cofactor. This%%%
%%% activity could be selectively suppressed using low concentrations of%%%
%%% Mg2+ (less than 1 mM), thus allowing nicked double-stranded circular%%%
%%% DNA to be gapped to a single-stranded circular species. The enzyme may%%%
%%% be useful for purifying double-stranded closed circular DNA from%%%
%%% nicked, linear or single-stranded contaminants in site-directed%%%
%%% mutagenesis, or for %%%plasmid%%% %%%DNA%%% purification from sheared%%%
%%% genomic DNA contaminants. (23 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/38 (Item 27 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0121883 DBA Accession No.: 91-09525%%%
%%%Cloning, sequencing, and characterization of the intracellular invertase%%%
%%% gene from Zymomonas mobilis - beta-D-fructofuranosidase production and%%%

%%% purification; expression in Escherichia coli; DNA sequence%%%
%%%AUTHOR: Yanase H; Fukushi H; Ueda N; Maeda Y; Toyoda A; Tonomura K%%%
%%%CORPORATE SOURCE: Department of Biotechnology, Faculty of Engineering,%%%
%%% Tottori University, Tottori 680, Japan.%%%
%%%JOURNAL: Agric.Biol.Chem. (55, 5, 1383-90) 1991%%%
%%%CODEN: ABCHA6%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: The structural gene for the intracellular%%%
%%% beta-D-fructofuranosidase (BFF, EC-3.2.1.26) E1 of Zymomonas mobilis%%%
%%% Z6C was cloned in a 2.25 kb %%%DNA%%% fragment on %%%plasmid%%% pUSH11,%%%
%%% and expressed in Escherichia coli HB101. BFF produced by E. coli%%%
%%% carrying pUSH11 was purified about 1,122-fold to homogeneity with a%%%
%%% yield of 4% by ammonium sulfate precipitation, followed by column%%%
%%% chromatography on DEAE-Toyopearl 650M and CM-Sephadex C-50 and%%%
%%% concentration by %%%ultrafiltration%%% . The mol.wt. and substrate%%%
%%% specificity of BFF were identical with those of the intracellular%%%
%%% enzyme from Z. mobilis. The nucleotides of the cloned DNA were%%%
%%% sequenced; they included an open reading frame of 1,536 bp, coding for%%%
%%% a protein with a mol.wt. of 58,728. The N-terminal amino acid sequence%%%
%%% predicted was identical with the sequence of the 1st 20 N-terminal%%%
%%% amino acid residues of the protein obtained by Edman degradation.%%%
%%% Comparison of the predicted amino acid sequence of E1 protein with%%%
%%% those of 4 other known BFFs from E. coli, Bacillus subtilis, and%%%
%%% Saccharomyces cerevisiae indicated a stronger homology in the%%%
%%% N-terminal portion than in the C-terminal portion. (21 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/39 (Item 28 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0115893 DBA Accession No.: 91-03535 PATENT%%%
%%%L-lactate-dehydrogenase gene from Bacillus sp. - gene cloning and%%%
%%% expression in Escherichia coli using vector %%%plasmid%%% pTC1;%%%
%%% %%%DNA%%% sequence%%%
%%%PATENT ASSIGNEE: Toyama-Chem. 1990%%%
%%%PATENT NUMBER: JP 2286077 PATENT DATE: 901126 WPI ACCESSION NO.:%%%
%%% 91-012213 (9102)%%%
%%%PRIORITY APPLIC. NO.: JP 89108432 APPLIC. DATE: 890427%%%
%%%NATIONAL APPLIC. NO.: JP 89108432 APPLIC. DATE: 890427%%%
%%%LANGUAGE: Japanese%%%
%%%ABSTRACT: Bacillus sp. TP262 can grow at 50-70 deg, does not liquefy%%%
%%% gelatin and does not hydrolyze starch. It is a thermophilic bacterium%%%
%%% that produces L-lactate-dehydrogenase (LDH, EC-1.1.1.27). A DNA%%%
%%% fragment (3.9 kb) containing the LDH gene is obtained by HindIII%%%
%%% cleavage of strain TP262 DNA, and can be expressed in a host%%%
%%% microorganism when ligated into a suitable vector %%%plasmid%%%. The%%%
%%% %%%DNA%%% sequence of the gene is provided in the specification. In an%%%
%%% example, TP262 cell extract was subjected to ammonium sulfate%%%
%%% precipitation (40%) and the supernatant was adsorbed onto a Butyl%%%
%%% Toyopearl 650S column, eluting with 20% saturated (NH4)2SO4-0.1 M%%%
%%% phosphate buffer. The eluate was %%%ultrafiltered%%% and the fraction%%%
%%% containing crude enzyme was purified by DEAE-cellulose DE52 column%%%
%%% chromatography, %%%ultrafiltration%%%, AG-NAD affinity chromatography%%%
%%% and HPLC. Chromosomal DNA was inserted into plasmid pUC19, and the%%%
%%% resulting plasmid was introduced into Escherichia coli JM103 to obtain%%%
%%% LDH-producing transformant TC1. Plasmid pTC1, carrying the LDH gene,%%%
%%% was obtained from TC1 and used to construct plasmid pTC17. LDH produced%%%
%%% by TC1 has been purified. (16pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/40 (Item 29 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0108800 DBA Accession No.: 90-11491%%%
%%%Partial characterization of Pseudomonas fluorescens subsp. cellulosa%%%
%%% endoglucanase activity produced in Escherichia coli - recombinant%%%
%%% cellulase production using new vector plasmid pPFC4; enzyme isolation%%%
%%% and purification; glucose or cellulose culture medium%%%
%%%AUTHOR: Wolff B R; Lewis D; Pasternak J J; +Glick B R%%%
%%%CORPORATE SOURCE: Department of Biology, University of Waterloo, Waterloo,%%%
%%% Ontario, Canada N2L 3G1.%%%
%%%JOURNAL: J.Ind.Microbiol. (5, 2-3, 59-64) 1990%%%
%%%CODEN: JIMIE7%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: Recombinant plasmid pPFC4 (a derivative of plasmid pBR322), which%%%
%%% carries Pseudomonas fluorescens subsp. cellulosa NCIB 10462 chromosomal%%%
%%% DNA was isolated for its ability to express cellulase complex%%%
%%% (EC-3.2.1.4) in Escherichia coli HB101, grown on M9 medium with 0.5 g/l%%%
%%% casamino acids and no additional C-source or with 1% glucose or 0.2%%%
%%% CM-cellulose. The plasmid was characterized physically and chemically.%%%
%%% Most of the cellulase (78.4%) activity was located in the periplasmic%%%
%%% space of E. coli. The plasmid-encoded cellulase had optimum activity at%%%
%%% pH 6 and 50 deg. With CM-cellulose-zymograms, after PAGE, periplasmic%%%
%%% extracts obtained by %%%ultrafiltration%%% of E. coli cultures carrying%%%
%%% plasmid pPFC4 exhibited 6 bands of cellulase activity. The mol.wt. of%%%
%%% the cellulase band was 29,000, while the remaining cellulase complex%%%
%%% bands had mol.wt. ranging from 48,000-100,000. The %%%DNA%%% insert of%%%
%%% %%%plasmid%%% pPFC4 was not large enough to contain 6 separate genes.%%%
%%% Therefore, the cellulase complex may result from post-translational%%%
%%% modification of 1 or 2 primary gene products. (30 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/41 (Item 30 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%

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%%%%%%%
%%%0099974 DBA Accession No.: 90-02665 PATENT%%%
%%%Recombinant human neutrophil chemotactic factor - gene cloning and%%%
%%% expression in Escherichia coli; %%%DNA%%% sequence; %%%plasmid%%%%%%
%%% pHNP101 vector construction%%%
%%%PATENT ASSIGNEE: Dainippon-Pharm. 1989%%%
%%%PATENT NUMBER: WO 8910962 PATENT DATE: 891116 WPI ACCESSION NO.:%%%
%%% 89-356492 (8948)%%%
%%%PRIORITY APPLIC. NO.: US 189164 APPLIC. DATE: 880502%%%
%%%NATIONAL APPLIC. NO.: WO 89JP437 APPLIC. DATE: 890426%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: A process for production of a human neutrophil chemotactic factor%%%
%%% (NCF) polypeptide is claimed, comprising culturing recombinant cells%%%
%%% containing an expression vector with a specific DNA sequence. A cDNA%%%
%%% encoding the human NCF is isolated from human mononuclear leukocytes%%%
%%% using the known partial protein sequence. The gene is preferably%%%
%%% expressed in Escherichia coli, using plasmid pHNP101 as expression%%%
%%% vector, with a trp promoter to control transcription placed upstream of%%%
%%% the gene, and the recombinant polypeptide has a mol.wt. of 8400. The%%%
%%% recombinant protein may be purified from cells by a combination of%%%
%%% removal of nucleic acid, salting out, anion-exchange and/or%%%
%%% cation-exchange chromatography, %%%ultrafiltration%%% and gel%%%
%%% filtration chromatography. NCF, alone or in combination with%%%
%%% interleukin-1, attracts and activates neutrophils at bacterial%%%
%%% infection foci, inflammation sites and around malignant tumor cells.%%%
%%% The combination of NCF and interleukin-1 can be used for therapy of%%%
%%% bacterial infections or tumors. (26pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/42 (Item 31 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0091591 DBA Accession No.: 89-09582 PATENT%%%
%%%Production of tissue plasminogen-activator - by human fetus lung fibroblast%%%
%%% cell culture transformed with vector plasmid pSV3neo, containing the%%%
%%% SV40 virus T-antigen gene%%%
%%%PATENT ASSIGNEE: Snow-Brand-Milk-Prod. 1989%%%
%%%PATENT NUMBER: JP 1091779 PATENT DATE: 890411 WPI ACCESSION NO.:%%%
%%% 89-148139 (8920)%%%
%%%PRIORITY APPLIC. NO.: JP 87249713 APPLIC. DATE: 871005%%%
%%%NATIONAL APPLIC. NO.: JP 87249713 APPLIC. DATE: 871005%%%
%%%LANGUAGE: Japanese%%%
%%%ABSTRACT: A new process for producing tissue plasminogen-activator (tPA)%%%
%%% involves culturing human cells carrying a vector with the SV40 virus%%%
%%% T-antigen gene. More specifically, %%%plasmid%%% %%%DNA%%% is purified%%%
%%% from Escherichia coli HB101 containing vector plasmid pSV3neo,%%%
%%% including the SV40 virus T-antigen gene. The %%%plasmid%%% %%%DNA%%% is%%%
%%% digested with EcoRI and treated with phenol and chloroform. The linear%%%
%%% DNA is precipitated with ethanol overnight, dried and dissolved in 1 mM%%%
%%% Tris-HCl containing 0.1 mM EDTA. Normal human fetal lung fibroblasts,%%%
%%% preferably IMR90 cells, are cultured in Dulbecco's Modified Eagle%%%
%%% medium with 10% fetal calf serum, 100 U/ml benzylpenicillin, and 100%%%
%%% ug/ml streptomycin until they become 50-70% confluent. The cells are%%%
%%% collected and suspended in Dulbecco's iced phosphate buffered saline.%%%
%%% The %%%plasmid%%% %%%DNA%%% is added to the cell suspension which is%%%
%%% then subjected to electroporation. The transduced cells are cultured%%%
%%% and cloned after 2 wk. The culture solution is concentrated by%%%
%%% %%%ultrafiltration%%% , and treated with anti-tPA monoclonal antibody%%%
%%% affinity chromatography. The resultant tPA is purified by ethanol%%%
%%% precipitation and HPLC. (7pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/43 (Item 32 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0088299 DBA Accession No.: 89-06290 PATENT%%%
%%%Production of human fibroblast recombinant interferon - by Chinese hamster%%%
%%% ovary cell culture transformed with plasmid pSVDHFR and plasmid pSVEIF%%%
%%%PATENT ASSIGNEE: Yeda-Res.Develop. 1989%%%
%%%PATENT NUMBER: US 4808523 PATENT DATE: 890228 WPI ACCESSION NO.:%%%
%%% 89-085029 (8911)%%%
%%%PRIORITY APPLIC. NO.: US 669259 APPLIC. DATE: 841107%%%
%%%NATIONAL APPLIC. NO.: US 669259 APPLIC. DATE: 841107%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: A new CHO cell culture, designated CHO-beta-1-5-9 (Pasteur%%%
%%% Institute Order No. I-340), is resistant to more than 50 nM%%%
%%% methotrexate. CHO-beta-1-5-9 contains plasmid pSVDHFR, carrying a%%%
%%% selectable marker, and plasmid pSVEIF, carrying a sequence encoding%%%
%%% human fibroblast interferon beta-1 (IFN-beta-1-) fused about 60 bp down%%%
%%% stream from the SV40 virus early start gene. CHO-beta-1-5-9- can be%%%
%%% cultivated for secretion of an IFN-beta-1- glycoprotein into the%%%
%%% culture medium, giving yields greater than 50,000 units/1,000,000%%%
%%% cells/24 hr. More specifically, CHO-beta-1-5-9 cells are grown at 37%%%
%%% deg on microcarrier beads comprising glass, plastic or another%%%
%%% polymeric substance with periodic replacement of culture medium,%%%
%%% preferably every 24 hr. Suitable culture media include Dulbecco's%%%
%%% modified minimal essential medium containing 150 ug/ml proline and 1%%%%
%%% fetal calf serum. The collected medium is: applied to an affinity%%%
%%% chromatography adsorbent; eluted; concentrated by %%%ultrafiltration%%%%%%
%%% ; subjected to affinity chromatography against monoclonal antibodies%%%

%%% prepared against IFN-beta-1 from human fibroblasts; and homogeneous%%%
%%% IFN-beta-1 is collected. (14pp)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/44 (Item 33 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
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%%%%%%%
%%%0084896 DBA Accession No.: 89-02887%%%
%%%Cloning and expression of B. amyloliquefaciens secretory metallo-protease%%%
%%% npr gene in B. subtilis cells - Recombinant Bacillus amyloliquefaciens%%%
%%% metallo protease production, isolation and purification%%%
%%%AUTHOR: Jomantas J V; Gervinskas V V; Kozlov Y I; Anukhin Y M; Sterkin%%%
%%% V E; Izotova L S%%%
%%%CORPORATE SOURCE: All-Union Research Institute of Genetics and Selection of%%%
%%% Industrial Microorganisms, Moscow, USSR.%%%
%%%JOURNAL: Biotekhnologiya (4, 6, 692-98) 1988%%%
%%%CODEN: BTKNEZ%%%
%%%LANGUAGE: Russian%%%
%%%ABSTRACT: Bacillus amyloliquefaciens metallo protease gene was cloned and%%%
%%% expressed in Bacillus subtilis. B. amyloliquefaciens chromosomal DNA%%%
%%% was digested with EcoRI and ligated with linearized %%%DNA%%% from%%%
%%% %%%plasmid%%% pJJ2 (a 2-replicon shuttle vector), and the ligation%%%
%%% mixture was used to transform B. subtilis strain 1025 (defective in the%%%
%%% production of secreted alpha-amylase, alkaline protease and metallo%%%
%%% protease). A casein-hydrolyzing clone was identified and recombinant%%%
%%% plasmid pNM1 was isolated from it. A BglII-BclI DNA fragment including%%%
%%% the npr gene from plasmid pNM1 was recloned in plasmid pUB110. A%%%
%%% plasmid pNP6, carrying a 1.9 kb B. amyloliquefaciens DNA sequence and%%%
%%% the npr gene, was isolated. This plasmid was stably inherited by B.%%%
%%% subtilis AJ73 cells over at least 20 generations. The recombinant%%%
%%% strain produced only 1 protein, a metallo protease, which was secreted%%%
%%% at 200-300 mg/l protein. The enzyme was isolated from the culture%%%
%%% medium by %%%ultrafiltration%%%, acetone precipitation, gel-filtration%%%
%%% and ion-exchange chromatography on CM-cellulose or affinity%%%
%%% chromatography on bacitracin-Sepharose. (15 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/45 (Item 34 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
%%%(c) 2001 Derwent Publ Ltd. All rts. reserv.%%%
%%%%%%%
%%%0079761 DBA Accession No.: 88-10610%%%
%%%A general method of in vitro preparation and specific mutagenesis of DNA%%%
%%% fragments: study of protein and DNA interactions - using the polymerase%%%
%%% chain reaction%%%
%%%AUTHOR: Higuchi R; Krummel B; Saiki R K%%%
%%%CORPORATE AFFILIATE: Cetus%%%
%%%CORPORATE SOURCE: Department of Human Genetics, Cetus Corporation,%%%
%%% Emeryville, CA 94608, USA.%%%
%%%JOURNAL: Nucleic Acids Res. (16, 15, 7351-67) 1988%%%
%%%CODEN: NARHAD%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: Specific, end-labeled DNA fragments were prepared using a%%%
%%% polymerase chain reaction (PCR). %%%Plasmid%%% %%%DNA%%% from a%%%
%%% mini-preparation was used as template for DNA amplification. 1 Primer%%%
%%% was 32-P-labeled at its 5' end, giving an end-labeled product. PCR was%%%
%%% carried out by mixing Thermus aquaticus DNA-polymerase (EC-2.7.7.7),%%%
%%% buffer, dNTPs and primers followed by temperature cycling, causing%%%
%%% repeated DNA denaturation (94 deg), primer annealing (37 deg), and%%%
%%% primer extension (72 deg), amplifying DNA sequences flanked by the%%%
%%% primers. The final yield of amplified DNA was 2-5 pmol/0.1 ml PCR.%%%
%%% PCR-labeled blunt-ended DNA fragment had identical protection patterns%%%
%%% to end-labeled restriction fragments in DNA-ase I protection footprint%%%
%%% assays, and were used for chemical sequencing and production and%%%
%%% analysis of paused RNA-polymerase (EC-2.7.7.6) transcription complexes.%%%
%%% Site-directed mutagenesis of PCR fragments was carried out by%%%
%%% recombining overlapping mutated PCR fragments and reamplifying the%%%
%%% product. Insertions, deletions or base substitutions could be%%%
%%% introduced at any position. Excess primers were removed rapidly by%%%
%%% %%%ultrafiltration%%%. (18 ref)%%%
%%%%%%%
%%%%%%%
%%% 8/3,AB/46 (Item 35 from file: 357)%%%
%%%DIALOG(R)File 357:Derwent Biotechnology Abs%%%
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%%%%%%%
%%%0076043 DBA Accession No.: 88-06892%%%
%%%%%%%Ultrafiltration%%% for the removal of excess DNA linkers subsequent to%%%
%%% ligation - analysis of EcoRI linker removal by cloning using plasmid%%%
%%% pIBI and Escherichia coli transfection%%%
%%%AUTHOR: Miller J S%%%
%%%CORPORATE SOURCE: Dept. of Biological Chemistry, Harvard Medical School, 25%%%
%%% Shattuck Street, Boston, MA 02115, USA.%%%
%%%JOURNAL: BioTechniques (5, 7, 632-34) 1987%%%
%%%CODEN: BTNQDO%%%
%%%LANGUAGE: English%%%
%%%ABSTRACT: Linkers are useful for inserting fragments of DNA efficiently%%%
%%% into a vector, and allow easy removal of the fragment, once cloned, and%%%
%%% allow insertion of desired sequences at a specific site in DNA. EcoRI%%%
%%% (EC-3.1.23.13) linkers were phosphorylated and reaction mixture%%%
%%% containing linker was diluted and centrifuged. The%%%
%%% %%%ultrafiltration%%% device used was a Centricon-30 microconcentrator%%%
%%% (Amicon). The concentrate was diluted and centrifuged, and the process%%%
%%% was repeated once more. %%%Plasmid%%% pIBI %%%DNA%%% was digested with%%%
%%% EcoRI and dephosphorylated with calf intestinal phosphatase. It was%%%
%%% purified from an agarose gel. Reaction mixture starting material and%%%
%%% aliquots from each concentration stage were incubated with pIBI with T4%%%
%%% DNA-ligase in ligation buffer. Next day, the ligation reaction mixture%%%
%%% was transfected into Escherichia coli HB101 competent cells.

Colonies%%%
%%% were examined for presence of cloned linkers. Linkers were
effectively%%%
%%% removed after the 3rd centrifugation. (4 ref)%%%
%%%? log%%%